VITAMINS

A Digest of
Current Knowledge

By the same author—

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(*With E. Baldwin and others*)

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VITAMINS

A Digest of Current Knowledge

By

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(Medical Research Council and University of Cambridge)

With 84 illustrations
111 structural formulæ



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PREFACE

The object of this book is to supply a relatively brief and summarized account of all the more important known facts and conclusions about the vitamins

It is hoped that the book may be useful to University science students in the earlier stages of their career and to others who may wish to learn about current knowledge and views

As to the scope of the book, I have included in the biochemical sections for example, the greater part of the ground covered in my lectures to the Part II Tripos Class in Biochemistry in Cambridge (i e the Advanced Honours Course) The treatment is somewhat fuller than that needed for Part I of the Honours Course, and it may therefore be regarded as a compromise between these two stages. The sections dealing with the chemical, the pathological and the medical aspects, on the other hand are rather more detailed than is required for those whose interests are exclusively biochemical and these sections should meet the corresponding needs of students in these other subjects, at the same stages of their work.

I have tried to bear in mind also the requirements of other classes of readers e g medical men chemists, dietitians, who may have occasion to consult such a book as this in order to refresh their memories or to gain an impression of the present state of knowledge in some particular direction Also I hope that the more advanced student or research worker may possibly find it useful to take the summarized statements and brief lists of references given in this book as the starting point for his more extended bibliographical research.

The object in the bibliography has generally been t
cite the earliest reference in the literature to the topi
under discussion but quite often I have considered
helpful to add references to a recent review article an
in some instances a fuller bibliography is provided for
matter of special topical interest.

I am a firm believer in the value of the historic
approach for anyone wishful of gaining a true unde
standing of and insight into any scientific question an
I therefore make no apology for my devotion to it here.

The present volume may in a sense be regarded as
condensed version of my larger work, *Vitamins and Vitam*
Deficiencies Vol. 1 of which was published in 1938 an
the printing of the remainder of which had to be pos
poned because of the outbreak of World War II with i
consequent famine in newsprint, and its other preoccupa
tions. The task of revising the typescript of the large
work has become a formidable undertaking and in th
meantime this briefer synopsis is sent out in the hope tha
it may perhaps meet some of the same needs. My presen
text is derived in large measure from review article
based in turn on my larger work, and contributed to suc
publications as *The British Encyclopædia of Medical Practic*
Chambers's Encyclopædia and *Thorpe's Dictionary of Applie*
Chemistry—and to all of these I am grateful for permissio
to reproduce here.

In preparing this book, I have had the inestimabl
advantage of being able to submit the proofs to a numbe
of eminent experts and specialists, including Mr A. I
Bacharach Dame Harriette Chick, Dr S K. Kon, Pro
J R. Marrack, and Sir Charles Martin and if the tex
is found to be relatively free from errors and not unfa
in its balance of treatment, a large measure of the credi
is certainly due to these friends for their kindly comments
criticisms and corrections. I am no less indebted in th

same direction, to a number of my own colleagues at Cambridge, including Dr V H Booth Dr I Clark, Miss E M Cruickshank, Dr D A G Galton Mr M B Harris, Dr E Kodicek and Dr Thomas Moore, and to them too I wish to express my sincere thanks

At the suggestion of several friends, I have appended a Glossary of Medical Terms in the hope that it may prove of use to readers whose approach to the subject is on the scientific side rather than the clinical

The preparation of the typescripts would not have been possible without the loyal and skilful secretarial assistance given by Miss J E. Etchells and Miss A C Martin In the drawing and photographing of the structural formulæ I have had the expert and careful technical assistance of Mr A Ward and Miss N L. Gass

LESLIE J HARRIS

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CONTENTS

The fuller account of the discovery of vitamins, and of their differentiation from one another will be found in Chapter II

Definition

It is not easy to give a completely satisfactory definition of what we understand by the term vitamin but the following is probably as good as any —

Vitamins are substances that (a) are distributed in foodstuffs in relatively minute quantities that (b) are distinct from the main components of food (i e proteins, carbohydrates, fats mineral salts and water) that (c) are needed for the normal nutrition of the animal organism, and (d) the absence of any one of which causes a corresponding specific deficiency disease

What this definition implies will be more clearly understood as we expand it, point by point, in the following pages *

The Vitamins Enumerated

At present (1951) about **20 vitamins** are known. These are listed in Appendices I and II At least **eight** of

* *According to this definition,* ascorbic acid (vitamin C) is a vitamin for a human being or guinea pig, but not for a rat, since the rat can synthesize it in its body and hence does not need it in its diet. Again, a vitamin may be needed in one set of circumstances and not in another for example, a supply of vitamin D is no longer necessary in the diet if the body is exposed to ultra violet irradiation (p. 100)

p-Aminobenzoic acid is a vitamin (e.g for the rat) since, although it is an amino-acid, it is not one of the amino-acids normally occurring in proteins. On the other hand, the so-called *nutritionally essential amino-acids* (viz tryptophan, lysine, etc.) are not vitamins, since they are part-and-parcel of the protein molecule. Logically "vitamin F" (the group of nutritionally essential, unsaturated fatty acids) should be excluded since it does not comply with clause *b* but it fulfils all the other criteria, and for practical convenience is best discussed with (if not strictly speaking included with) the vitamins.

Choline is needed (by a rat) at the level of about 0·1% of the diet, a larger requirement than, for example, for vitamin D (0·000002%) or vitamin B_1 (0·0003%) but this does not prevent it from qualifying under clause *a*.

them are known to be **needed by man** or are of undoubted clinical significance namely —

Vitamin A
Vitamin B_1
Nicotinamide, Riboflavin, Folic acid — components of "vitamin B_2 complex."*
Vitamin C
Vitamin D
Vitamin K

Vitamin D for example, is the factor which protects against **rickets** a common ailment of childhood (Chapter VI) Vitamin C prevents **scurvy** a disorder formerly prevalent among sailors and due to insufficient use of fresh fruits and vegetables (Chapter V) Vitamin B_1 deficiency is the cause of **beri beri** a disorder widespread in rice-eating countries in the East (Chapter III)

There are **seven** vitamins whose exact **significance for human nutrition** still remains uncertain namely —

Pyridoxin, Pantothenic acid, Biotin, Choline — B_2 vitamins
Vitamin E
Vitamin F
Vitamin P

and another **three** (all classed as B_2 factors) which have so far been demonstrated only to be **needed by animals or by micro organisms** namely —

Inositol
p-Aminobenzoic acid
Strepogenin

* For explanation of "B_2" see pages 6 47

As this book is being written new **vitamins** are s coming to light, including—a very important addition

Vitamin B_{12},

and also a so-called

Animal protein factor (or factors)

Most of the vitamins exist in **more than one form** **modification** For example, vitamin A_1 and β-carot are two of the more important forms of vitamin A, a vitamins D_2 and D_3 of vitamin D Most of the vitam have also been given more exact **chemical names** each of their several forms, and (unfortunately) so times several **synonyms**. Thus crystalline vitamin is called *calciferol* and vitamin B_1 is *thiamine* in Amer or *aneurin* in Europe Only one form of vitamin C known its chemical name being *ascorbic acid*

Distribution in Foods

Foods vary greatly in the amounts of the differ vitamins which they contain For example, halibut liv oil is extremely rich (and cod liver oil somewhat less ric in vitamins A and D but contains no trace of vitami B C or E Certain fruits—for example, oranges lemo and blackcurrants—are valuable sources of vitamin C b they contain little or no vitamins A or D Apples a plums, on the other hand are less good for vitamin Again, different types of apples vary in the amount of th vitamin which they contain Wheatmeal bread is good instance of a food well supplied with vitamin B but there is little in white flour white bread or biscui and these are all devoid of vitamins C and D

Milk is an example of a food which contains *all* t vitamins so far mentioned (and others in additio although it is richer in some of them than in others—th it contains relatively little vitamin E

It is scarcely ever possible to give an absolute value for the vitamin content of a food but only an **average value or range** For example, Seville oranges as purchased in Britain contain on the average around 35 mg of ascorbic acid per 100 g but individual specimens may contain anything from 20 to 45 mg Moreover a **variable degree of destruction** of the different vitamins may occur as a result of ageing storage, handling processing and cooking of the foods This will depend on the conditions employed and on the vitamin in question For instance an average value for vitamin C in *new* potatoes is 45 mg per 100 g for the variety King Edward, and 25 mg for Majestic Old potatoes may contain only one third to one half as much as new potatoes Cooking by boiling may cause an average loss of about 60 per cent.

Details about the amount of each vitamin contained in typical foods will be given in the appropriate chapters and a summarized statement will be found in Appendix III

Vitamins and Human Health

A certain **minimum quantity** of each vitamin is needed in our diet if we are to be protected from the corresponding vitamin-deficiency disease. For example, a diet containing no fresh fruit or vegetables, and consisting only of such foods as dried fish dried meat bread and butter cakes and pastries, tea, cocoa sugar and condensed milk is devoid of vitamin C. An adult restricted to it would develop scurvy in about 6–9 months If however any food containing enough vitamin C to supply about 10–20 mg per day were to be included in this diet (or the same amount of the pure crystalline substance were to be administered) scurvy would be prevented or cured Similarly for the other vitamins

The **daily minimum dose** varies greatly from one vitamin to another It is usual to add a small margin for

safety and for human variability and with this proviso the **reputed daily requirements** range from 30 mg per day for vitamin C down to 1 mg for vitamin B_1, or 0–01 mg for vitamin D (pp 34, 77 105 and Appendix I)

The foregoing figures give the requirements in approximate, round numbers and it would be a mistake to apply them over rigidly Indeed the amount of a given vitamin needed may vary with other factors Thus the requirement for vitamin B_1 depends on the carbohydrate intake, and for vitamin D on the calcium and phosphate, and other things The value cited above for vitamin D applies to a child adults appear to need much less.

The type of diets consumed in normal circumstances by most middle-class adults in Britain as well as in most parts of Europe and America for example, can be relied on to *supply a sufficiency of all the vitamins provided* that it is reasonably varied (a good mixed diet) This is not to say that vitamins are, after all of little practical significance. Their discovery has been of immense consequence, and has made possible the conquest of diseases such as rickets formerly very common indeed in Britain, or of scurvy beri beri and pellagra, scourges in other regions of the globe. Indeed rickets still occurs in Britain U S.A. and elsewhere, because of a neglect to supplement children s diets with adequate vitamin D

Sometimes a vitamin-deficiency disease may result not because of a deficiency in the diet, but because the patient is unable to ingest or absorb the vitamin This is called a **conditioned deficiency** Examples are the polyneuritis (=beri beri) or the pellagra like symptoms that are from time to time found associated with gastro-intestinal obstruction or with alcoholism and which are caused by the inability of the patient to ingest (or to utilize or assimilate) the vitamin B_1 or nicotinamide, respectively Similarly a deficiency of vitamin K,

manifest as a hæmorrhagic disease (p 168) is generally conditioned by failure of absorption

Chemical Properties and Functions of the Vitamins

In the early days of vitamin research a somewhat arbitrary but useful distinction was made between the **fat soluble** and the **water soluble** vitamins The distinction is based on the fact that the former are found to occur in fatty foods only or in the fatty portions of food and the latter in the non fatty foods or fractions Similarly the fat-soluble vitamins dissolve in the so-called *fat solvents* (e.g ether light petroleum) while the water-soluble vitamins are generally soluble in various *aqueous media* including dilute acids and mixtures of alcohol and water Five of the vitamins are fat-soluble viz. —

Vitamin A
Vitamin D
Vitamin E
~~Vitamin F~~
Vitamin K

The remainder are water-soluble

When the vitamins are described one by one in the following chapters it will be found that they differ widely in their chemical nature, and possess few if any chemical features in common In one respect however namely in their biochemical mode of action several of the **water soluble vitamins** are recognized to function as **co enzymes** (see for example pp 41 69) On this basis an alternative definition of a vitamin may be sought in place of that given on p 2 namely that a vitamin is an **exogenous catalyst**—or one which the animal organism needs but is unable to synthesize and must therefore receive in its food

Requirements of Different Species

Species vary considerably in their needs for the different vitamins For example, human beings need daily approximately 0 02 mg of vitamin C for every 100 g of body weight but the guinea pig needs about forty times as much (i e. 0 8 *mg* per 100 g) *Monkeys also require* vitamin C, but other animals, including rats, mice, cats, dogs, horses, cattle and sheep do not need vitamin C in their diets, the reason being that they are able to synthesize it in their bodies (see footnote, p 2)

Again certain species particularly **ruminants** (including such farm animals as cattle and sheep) thrive in the absence of B vitamins from their diet, whereas other species such as humans rats and dogs, develop fatal deficiency diseases—beri beri pellagra, etc. The reason appears to be that the first mentioned group obtain these vitamins from the alimentary canal where they are **elaborated by micro organisms** particularly by those associated with the massive processes of fermentation which occur in the rumen

Several of the vitamins are known to play an important rôle in **plant** physiology Vitamin like substances needed specially by higher plants are named **auxins** Again vitamin like substances are needed by **bacteria** and other microbes ***(growth promoting factors for micro* organisms)** and these may or may not be vitamins for mammals.

Methods of Estimation

Three main types of assay are employed in determining the amount of a particular vitamin present in a foodstuff or animal or vegetable tissue. These are —

(a) **Chemical** or **physico chemical**
(b) **Biological**
(c) **Microbiological**

Rats are the species most commonly used for biological assay except that guinea pigs have to be used for vitamin C Pigeons, chicks, dogs pigs, and other species, have all been used for special purposes.

In the microbiological method the principle is to choose organisms which need the vitamin in question for their nutrition and to measure their growth or some metabolic activity

Examples of physico-chemical methods are the determination of vitamin A by its ultra violet absorption spectrum, and of carotene by its natural yellow colour (Chapter VII) A simple **chemical** method is the determination of vitamin A by its reaction with antimony trichloride, or of vitamin C with an indophenol derivative (Chapter V) **Microbiologically** nicotinamide or riboflavin can be assayed by measuring the growth or the lactic acid production in the organisms *Lactobacillus arabinosus* and *Lactobacillus casei* respectively (Chapters IV and IX)

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CHAPTER II

VITAMIN HISTORY

Recognition of the existence of vitamins has grown gradually The first step in a long series of developments leading eventually to the vitamin(e) hypothesis may be discerned in some early observations on deficiency diseases in man especially on scurvy and beri beri *

Empirical Cures of Scurvy Beri beri and Rickets

We have to go back to the sixteenth century for what appear to be the first records of the cure of scurvy—that old scourge of mariners—by such agents as a decoction of spruce needles (Jacques Cartier 1535) or oranges and lemons (Sir Richard Hawkins, 1593) It was early in the seventeenth century (1601) that Sir James Lancaster introduced the regular use of oranges and lemons into the ships of the East India Company as a preventive against the disease Many others during the seventeenth and eighteenth centuries repeatedly confirmed the fact that fresh fruits and vegetables were effective in curing or preventing scurvy (e g Woodall 1639 Kramer 1739 Lind 1757 Captain James Cook, 1772) In 1804 the daily consumption of lemon juice was made compulsory in the British Navy

In 1882 Takaki found that he could stamp out beri beri in the Japanese Navy by certain changes in the diet

* For a more detailed history of the discovery of vitamins, see Harris (1935, 1938)

He considered however that it was an increase in the protein intake which was responsible for the cure (see Takaki 1885 etc)

Thus we may say that although dietary cures had been discovered for the control of these two diseases—beri beri and scurvy—yet the true nature of the dietary error responsible for them remained unknown It may nevertheless be recalled in parenthesis that a far sighted physician named Budd had predicted in 1840 that scurvy is due to the lack of an essential element which it is hardly too sanguine to state will be discovered by organic chemistry or the experiments of physiologists in a not too distant future

Towards the end of the nineteenth century the view began to be expressed by pædiatricians that rickets is produced as certainly by a rachitic diet as scurvy by a scorbutic diet (Cheadle, 1899)

Discovery of Experimental Avitaminoses

In 1890 Eijkman in the Dutch East Indies made the important discovery of **experimental beri beri** in fowls From 1890 to 1897 he carried out the earliest work on the extraction of the antineuritic substance (now called vitamin B_1) which he found to be present in the bran of rice but not in polished (milled) rice But the first to state clearly that beri beri was due solely and simply to a dietary deficiency and was not caused by any positive agent or toxin was Eijkman s collaborator Grijns (1901)

In 1907 Holst and Frolich in Christiania [now Oslo] discovered **experimental scurvy** in guinea pigs. With Eijkman s pioneer work on beri beri in mind they rightly considered this to be likewise a deficiency disease and set out to examine the properties of the anti-scorbutic substance (now vitamin C)

The Concept of Vitamins

By 1906 Hopkins could refer to scurvy and rickets as diseases in which for long years we have had knowledge of a dietetic factor He realized moreover that the errors in the diet "although still obscure were certainly of the kind which comprises the minimal qualitative factors In 1912 Funk, then working on the anti beri-beri factor (Chapter III), propounded his 'vitamine theory—i e. he postulated the existence of separate anti-beri beri, anti scurvy anti rickets and anti-pellagra vitamines

Experiments on Synthetic Diets '

In the meantime, experiments had been in progress attacking the problem from an entirely different angle—investigating not deficiency diseases as such, but determining what constituted a physiologically complete diet. Lunin a pupil of the Swiss biochemist Bunge, first showed in 1881 that animals failed to thrive when kept on an artificial regimen comprising the then known constituents of food i.e. re-purified fat, protein carbohydrate, mineral salts, and water He concluded that a *natural food such as milk must therefore contain* besides these known principal ingredients small quantities of other and unknown substances essential to life Similar conclusions were reached by various other workers, of whom the most notable were Socin (1891) and Stepp (1909) mention must also be made of Coppola (1890) Hall (1896) Häusermann (1897) Henriques and Hansen (1905) Falta and Nœggerath (1905) and Jacob (1906) The eminent Dutch physiologist Pekelharing published in 1905 the statement—generally overlooked at the time—that the unknown substances must be effective in very minute amounts, for he had found that quite small supplements of natural foods added to the artificial

synthetic ration were sufficient to afford protection An independent and more detailed study by Hopkins (1912) had as its principal conclusions (i) that an insignificantly small addition of milk would suffice to render the purified

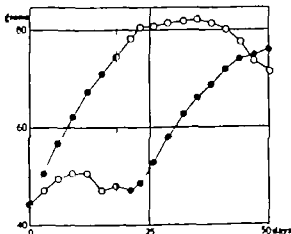


Fig. 1 Growth curves of rats, with and without supplement of milk (Hopkins, 1912)

○ ○ ○ Basal diet alone (purified protein, fat, carbohydrate, mineral salts, water)

● ● ● Basal diet plus small addition of milk

diet adequate and (ii) that the animals ceased to grow while still eating sufficient *in quantity* of the purified diet to support good growth

The Modern Period

The year 1912 may be said to mark the beginning of modern intensive work on vitamins. Hopkins's celebrated paper and Funk's review published a few months earlier for the first time attracted world wide attention to the vitamin question For a few years longer nevertheless the existence of vitamins was still disputed

Chemical Names and Chemical Relations

Vitamin A ($C_{20}H_{29}OH$) was shown to be chemically related to the naturally occurring hydrocarbon β-carotene ($C_{40}H_{56}$) which shares its biological activity Vitamin B_1 ($C_{12}H_{17}N_4OSCl.HCl$) has been given the name of aneurin (or thiamine in U S.A) Vitamin C ascorbic acid has the formula $C_6H_8O_6$ There are several forms of vitamin D the most important being calciferol or 'vitamin D_2 ($C_{28}H_{43}OH$) and vitamin D_3 ($C_{27}H_{43}OH$) Vitamin E α tocopherol has the formula $C_{29}H_{50}O_2$ there are also β- γ- and δ-tocopherols with somewhat lower biological potency (for rats) Chemical names and synonyms for other vitamins are given else where in the text.

Further Progress

Side by side with these investigations on the organic chemistry of the vitamins, methods were being elaborated by which vitamins could be accurately assayed in bio-logical materials or in foodstuffs, and the losses incurred on cooking or processing ascertained their chemical and physical properties were being examined in close detail tests were devised to assess the status of a human subject in some particular vitamin the daily requirements were being estimated some new and unexpected clinical uses were found and the mode of action of the vitamins in the body was being gradually elucidated

A notable advance in this last mentioned direction was the discovery that various vitamins of the B group owe their biological activity to the fact that they can function as **co enzymes** in the living cell (cf p 7 and pp 44 63 175, 178 180) The pioneer observation of this kind was the finding of Lohmann and Schuster in 1937 that the pyrophosphate ester of vitamin B_1 is the co-enzyme for decarboxylation of **pyruvic acid** a substance which is

an important metabolite in the breakdown of carbohydrates

Recapitulation

The early stages of vitamin history are summarized in Tables 1 and 2 For a fuller catalogue of the vitamins now known reference may be made again to Appendices I and II

TABLE 1

Chronological Chart

The Early History of Vitamins

Part I Deficiency Diseases.

I Man		
601	**Scurvy** (Lancaster)	Prevented *empirically* by dietary additions.
1882	**Beri beri** (Takaki)	
1900 ca	**Rickets**	
In Experimental Animals		
1890-7	EIJKMAN	Experimental beri-beri discovered. First work on anti-beri-beri factor
901	GRIJNS	Beri-beri simply deficiency disease.
1907 2	HOLST and FROELICH	Experimental scurvy Scurvy similarly deficiency disease. Work on anti-scurvy factor
Theory		
1840	BUDD	Anti-scorbutic factor postulated.
1906	HOPKINS	Scurvy and rickets— Minimal qualitative factors
9	FUNK	The "vitamine theory Anti-beri-beri, anti-scurvy anti-rickets and anti-pellagra "vitamines postulated

Part II Normal Diets.

1881	LUNIN	Purified basal diets inadequate
{1909	*STEPP*	*Extracted* bread and milk *inadequat*
{1905	PEKELHARING	*Small* supplement of milk suffices.
9	HOPKINS	Convincing quantitativ evidence for these accessory factors.
1915	MCCOLLUM and DAVIS	Two such factors t least.

Chemical Names and Chemical Relations

Vitamin A ($C_{20}H_{29}OH$) was shown to be chemically related to the naturally occurring hydrocarbon β-carotene ($C_{40}H_{56}$) which shares its biological activity Vitamin B_1 ($C_{12}H_{17}N_4OSCl\ HCl$) has been given the name of aneurin (or thiamine in U S.A) Vitamin C ascorbic acid has the formula $C_6H_8O_6$ There are several forms of vitamin D the most important being calciferol or vitamin D_2 ($C_{28}H_{43}OH$) and vitamin D_3 ($C_{27}H_{43}OH$) Vitamin E α tocopherol, has the formula $C_{29}H_{50}O_2$ there are also β- γ- and δ-tocopherols with somewhat lower biological potency (for rats) Chemical names and synonyms for other vitamins are given else where in the text

Further Progress

Side by side with these investigations on the organic chemistry of the vitamins methods were being elaborated by which vitamins could be accurately assayed in biological materials or in foodstuffs; and the losses incurred on cooking or processing ascertained their chemical and physical properties were being examined in close detail tests were devised to assess the status of a human subject in some particular vitamin the daily requirements were being estimated some new and unexpected clinical uses were found and the mode of action of the vitamins in the body was being gradually elucidated

A notable advance in this last mentioned direction was the discovery that various vitamins of the B group owe their biological activity to the fact that they can function as **co enzymes** in the living cell (cf p 7 and pp 44 63 175, 178 180) The pioneer observation of this kind was the finding of Lohmann and Schuster in 1937 that the pyrophosphate ester of vitamin B_1 is the co-enzyme for decarboxylation of **pyruvic acid** a substance which is

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Recapitulation

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TABLE 1

Chronological Chart

The Early History of Vitamins

Part I Deficiency Diseases.

I Man		
1601	Scurvy (Lancaster)	Prevented *empirically* by dietary additions.
188	Beri beri (Takaki,	
1900 c.	Rickets	
In Experimental Animals		
1890-7	EIJKMAN	Experimental beri-beri discovered. First work on anti-beri-beri factor
190	GRIJNS	Beri-beri simply a deficiency disease.
1907 12	HOLST and FRÖLICH	Experimental scurvy Scurvy similarly a deficiency disease. Work on anti-scurvy factor
Theory		
184	BUDD	Anti-scorbutic factor postulated.
1906	HOPKINS	Scurvy and rickets— Minimal qualitative factors
1912	FUNK	The vitamine theory Anti-beri-beri, anti-scurvy anti-rickets and anti-pellagra "Vitamines postulated

Part II Normal Diets

188	LUNIN	Purified basal diets inadequat
{1909	STEPP	Extracted bread and milk inadequate
{1905	PEKELHARING	*Small* supplement of milk suffices.
912	HOPKINS	Convincing quantitativ evidence for these accessory factors.
19 5	McCOLLUM and DAVIS	Two such factors at least.

TABLE 2

Progress in Vitamin Chemistry

The Four Classical Deficiency Diseases

DEFICIENCY DISEASE	DISCOVERY OF PROTECTIVE FOODSTUFF	RECOGNITION OF A SPECIFIC PROTECTIVE FACTOR	VITAMIN ISOLATED (OR IDENTIFIED WITH SUBSTANCE PREVIOUSLY KNOWN*)	VITAMIN SYNTHESIZED (OR SYNTHESIZED BEFORE KNOWN TO BE A VITAMIN†)
Scurvy	Fruits and vegetables (ca. 1600)	1907	(1932*)	1933
Beri-beri	Whole rice, barley etc. (1882)	1901	1927	1936
Rickets	"Good fats" (ca. 1900)	1919-1922	1932	—‡
Pellagra	Good protein foods" (1916)	1926	(1937*)	(1867†)

‡Vitamin D_2 was prepared "artificially", by irradiation of the provitamin, ergosterol, in 1927. The "complete synthesis" has still to be accomplished.

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CHAPTER III

VITAMIN B_1 AND BERI-BERI

Vitamin B_1 deficiency was the first of the vitamin deficiencies to be produced experimentally in animals and to be recognized as such. It is, therefore, logical to begin the systematic study of vitamins with an account of beri-beri in experimental animals.*

BERI-BERI IN EXPERIMENTAL ANIMALS

Polyneuritis in Hens, Pigeons, etc.

As mentioned in Chapter II, polyneuritis in hens was discovered by Eijkman in 1890. Pigeons have been more

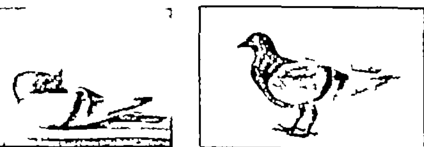


Fig. 3. Pigeon with polyneuritis (*left*) and after cure (*right*).
(*From photograph by Drummond*)

often used in later experimental work. After 12–24 days' restriction to a diet of polished rice, the birds become collapsed and helpless, and some of them may develop marked opisthotonos (retraction of the head) or some

* For a more comprehensive account of vitamin B and beri-beri, the books by Harris (1938), Williams and Spies (1938) and Cowgill (1934) may be consulted.

times emprosthotonos * Administration of vitamin B_1, or of a food rich in it, brings about a complete cure within a few hours. The very rapidity of the cure indicates that the nerve lesion is chemical rather than morphological Although, in advanced deficiency degenerative

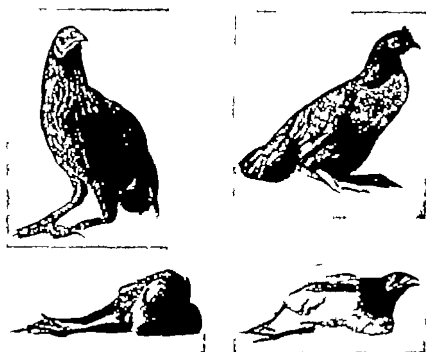


FIG 4. Fowls, with polyneuritis of increasing degrees of severity (After Fraser and Stanton)

changes may sometimes be found in the peripheral nerves these are not considered to be due specifically to lack of vitamin B_1

Polyneuritis has also been recorded in other species of birds kept on diets devoid of vitamin B_1 and some—e.g rice birds —have been used for purposes of biological assay

* See Glossary p. 221

In Rats

Rats are more conveniently used for experimental studies, and have the advantage over pigeons that they develop the symptoms and are cured, with greater regularity and reliability On a purified basal diet devoid of vitamin B_1 a state of acute deficiency (avitaminosis)



FIG. 5. Polyneuritic rat, with convulsions (*above*) and after cure (*below*)
(*After Smith and Mansell*)

marked by anorexia and progressive loss of weight, sets in after about three weeks. The characteristic polyneuritic symptoms are produced more effectively however by a chronic *partial* deficiency (hypovitaminosis) They include muscular incoordination with dragging of the hind limbs, and convulsions There is also a characteristic bradycardia (Drury and Harris, 1930 Drury Harris and Maudsley 1930)

In Other Species

Vitamin B_1 deficiency has been described also in mice, and various other animals A somewhat irregular

production of symptoms noted in some species (e.g cats, dogs) is possibly due to a variable degree of synthesis by micro-flora in the intestine (see next paragraph)

Micro synthesis in Ruminants Refection

In cattle, vitamin B_1 (as apparently also most of the other vitamins of the B group) is synthesized by the bacteria present in the rumen This is of practical importance since such farm animals are therefore independent of an external source of vitamin B_1

Sometimes in rats, particularly when their diet contains much raw starch a somewhat analogous process

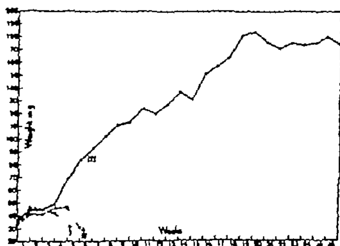


FIG. 6. Spontaneous refection in a rat (III) on a vitamin-B_1 free diet. Two other rats (I and II) remain un-refected, and die from the deficiency

(After Fridericia et al.)

involving excessive fermentation in the gastro-intestinal canal, called **refection** may occur spontaneously and prevent symptoms of deficiency occurring even when the diet is devoid of the vitamin (Fridericia 1926 Fridericia *et al* 1927)

Even in man a small variable amount of microsynthesis may occur (Najjar and Holt 1943)

Universal Need

Apparently vitamin B_1 is needed by all types of living matter including plants and micro-organisms, as well as by higher animals and insects for their vital processes Organisms differ however in the extent to which the vitamin must be supplied externally or to which it can be synthesized internally

BERI BERI IN MAN

Symptoms

Beri beri, common in the East among populations subsisting too exclusively on diets of polished (white) rice, is characterized by —

(1) **polyneuritis** (i e a multiple symmetrical peripheral neuritis* or neuropathy of which the typical manifestations include patches of paræsthesia in the lower limbs, diminished knee jerks wrist-drop high stepping gait muscular weakness)

(2) by **cardiac irregularities** (dyspnœa, tachycardia dilatation of the right side of the heart)

(3) anorexia and sometimes **emaciation** ("dry beri-beri") or at other times **œdema** ("wet beri beri")

The disease may follow several different courses depending on whether (1) the polyneuritic, (2) the cardiac, or (3) the œdematous symptoms predominate.

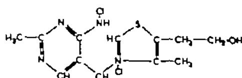
* Perhaps better described as a "neuropathy" (=disorder of nerves) than as a "neuritis" (=inflammation of nerves) since the morphological change (involving a myelin degeneration of the peripheral nerves) is not necessarily present in all cases and, as mentioned above the "lesion" may be regarded as essentially chemical.

Synonyms

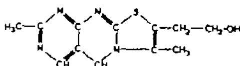
Vitamin B_1 was given the chemical name **aneurin** by Jansen, and was later renamed **thiamine** by Williams in America Both names are in use.*

Chemical Properties

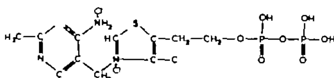
Vitamin B_1 is a base, forming salts, e.g the chloride-hydrochloride shown in the structural formula below



VITAMIN B_1



THIOCHROME



CO-CARBOXYLASE

Vitamin B_1 thiochrome and co-carboxylase

The fact that it is fairly readily destroyed by heat explains why it is absent from certain canned products or sterilized foods Alkalinity greatly increases the rate of destruction.

The vitamin is easily soluble in water and alcohol but *not in fat solvents.* It can be adsorbed on fullers earth and certain other substances The pulvis vitamini B_1 of an earlier British Pharmacopoeia was such an adsorbed

* Also two spellings, with or without the terminal -e are extant, *aneurin* or *aneurine* *thiamin* or *thiamine* *Thiamine* is now official in U.S.A., and *aneurin* in many British journals.

preparation supplying 100 units per gram in clinical practice it has since been superseded by the pure crystalline vitamin

Important derivatives of vitamin B_1 are

(1) **thiochrome** a substance devoid of antineuritic activity which is formed by mild oxidation with potassium ferricyanide in an alkaline medium and is used for the estimation of the vitamin (p 38) and

(2) the pyrophosphate ester **co-carboxylase** the form in which the vitamin functions biologically (p 41)

Anti vitamins etc.

Pyrithiamine, an artificially prepared pyridine analogue of aneurin can block the vitamin and thus operates as an **anti vitamin** Other substances antagonizing vitamin B_1 occur naturally in cereal grains, and in ferns. Thiaminase is the name for an enzyme found in raw fish which inactivates the vitamin by splitting it at the methylene bridge.

Distribution in Foods

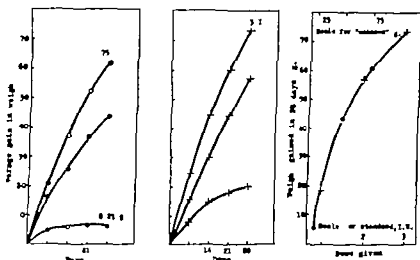
Vitamin B_1 is present, but in moderate amounts only in many natural foods The best sources include dried brewer s yeast (*ca* 1 000–2 000 i u per 100 g) wheat germ rice germ barley germ (*ca* 1 000) oatmeal (*ca* 300) wholemeal wheat flour peas haricot beans egg yolk (*ca* 100) White bread contains relatively small quantities. During the Second World War the most important measure for ensuring an adequate supply of vitamin B_1 for the population in Great Britain was the replacement of ordinary white flour (about 70–73 per

cent extraction) by the national wheatmeal flour (varying from about 80–85 per cent extraction) *

In animal tissues the vitamin occurs largely in the combined form as co-carboxylase, whereas in plant tissues it is largely as the free aneurin

Methods of Assay

The amount of vitamin B_1 in foods or tissues is commonly determined either by chemical test (e g by



1. Growth Curves. Graded doses of "unknown" 2. Growth Curves. Graded doses of standard. 3. Combined dose-response curves, "unknown" and standard.

FIG 19 Assay of vitamin B_1 by growth method with rats. (*After Harris*)

* *Explanation of "Extraction Rate of Flour"* True wholemeal flour ("hundred per cent") contains the whole of the wheat grain, i.e., endosperm, germ, and coarse and fine bran. Flour from which the coarsest of the particles of bran have been removed, and which consists of about 92 per cent of the original wheat ("92 per cent extraction") is popularly but inaccurately known as "wholemeal" By further removal of the finer bran, a flour which is almost white, representing 75 to 80 per cent of the grain, is obtained. To secure the whitest flour the extraction is reduced to 70–72 per cent, or even less.

In Great Britain, the extraction first was raised to 75 per cent in 1941 and then to 85 per cent in 1942 It fluctuated between 80 and 85 per cent until 1946, when it was increased to 90 per cent, and in the following year was reduced again to 85 per cent.

conversion into the fluorescent derivativ[e] or by biological assay) with rats—growth r[...] bradycardia or convulsions being the crite[...]

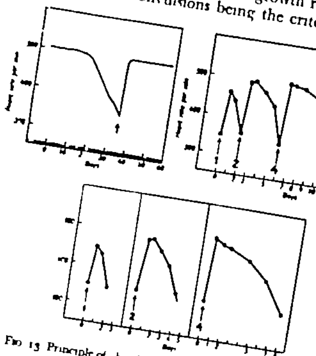
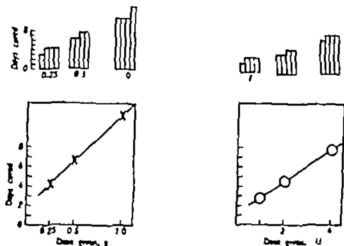


FIG 13 Principle of bradycardia test for assay of vitamin B[...]

Top left—Fall in heart rate in deficient rat, and cure on restoration of vitamin B_1 diet (*After Harris*[...])
Top right Duration of cure is proportional to size of dose (number[...] marked at arrow
Bottom—Same used repeatedly for successive doses

There is also a microbiological method depending o[...] stimulation of the rate of fermentation of yeast.*

Abridged literature guide for technique of methods of assay[...] (1 *Chemical thiochrome test* Jansen, 1936 Harris and Wang 194[...] 1948 Wang and Harris 1943 (2 *Chemical diazo test* Prebluda [...] McCollum, 1939 (3 *Bradycardia test* Birch and Harris, 1934; Le[...] and Harris 1937 Harris and Wang 194 (4) *Comparisons of ben[...] and biological results* Harris and Wang 194 (5) *Yeast fermentati[on] method* Schultz *et al* 1938.



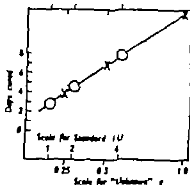


FIG 14. Assay of vitamin B$_1$ by bradycardia method.

(*After Harris*)

Above—Effects of graded doses of "test material" (*top left*) and "standard" (*top right*)

Right—Combined dose response curve, showing the relative activities of "test material" ("unknown") and "standard"

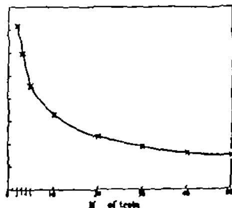


FIG. 15. Percentage accuracy of assay for vitamin B by bradycardia method.

The standard error of the assay is diminished in proportion to the number of replicate tests carried out (A "replicate test" = an individual dose given at any of the various levels of dosing of the "standard" or the "unknown")

(*After Leong and Harris*)

BIOCHEMICAL FUNCTION OF VITAMIN B_1

Relation to Carbohydrate Metabolism

It is now known that vitamin B_1 is concerned in the intermediate metabolism of carbohydrate. In animals or human beings suffering from a deficiency of the vitamin excessive amounts of lactic and pyruvic acids accumulate in the blood and tissues.* Administration of vitamin B_1 rectifies this metabolic error by accelerating the removal of these substances. Peters (1936) showed that added vitamin B_1 *in vitro* could restore the lost oxidative activity to avitaminous tissues respiring in the presence of lactic or pyruvic acids.

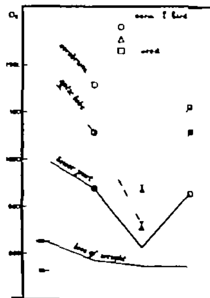


FIG. 6. Diminishing oxygen uptake by brain tissue of pigeons during development of avitaminous B_1 and rise on cure.

(*After Gavrilescu and Peters*)

Role as Co enzyme

The key to the puzzle was furnished when Lohmann and Schuster (1937) proved that the pyrophosphate ester of vitamin B_1 functions as the co-enzyme portion of the enzyme carboxylase.

Vitamin B_1 in this form (that is, as its pyrophosphate ester co-carboxylase) is needed for catalysing the decarboxylation and oxidation of pyruvic acid in animal

* Collazo, 1922; Bickel, 1924; Juno, 1926; Puglise, 1928; Hayasaka, 1930; Thompson and Johnson, 1934; Platt and Lu, 1935.

TABLE 4

*Examples of the Action of Co-carboxylase on Pyruvate in Different Systems**

Yeast

(1) SIMPLE DECARBOXYLATION

CH CO COOH (pyruvate) ⟶ CH_3 CHO + CO_2 (acetaldehyde)

Brain

EXAMPLES OF OTHER REACTIONS

(2) ANAEROBIC DISMUTATION AND DECARBOXYLATION

2CH CO COOH (pyruvate) + H_2O ⟶ CH COOH (acetate) + CH_3 CH(OH) COOH (lactate) + CO_2 —OXIDATION⟶ oxidation products

(3) OXIDATION AND DECARBOXYLATION

" + O_2 ⟶ 2CH COOH (acetate) + 2 CO

+ $5O_2$ ⟶ 6CO + 4 H O

Propionic bacteria, liver

(4) CARBOXYLATION (?)

pyruvate + CO_2 ⟶ oxalacetate —(+pyruvate)⟶ citric acid cycle ⟶ α-ketoglutarate + CO + fumarate (malate)

*Modified after Peters; Krebs; and others.

TABLE 5

Various Reactions in which Co-carboxylase Participates

(After Ochoa, 1942.)

REACTION	REFERENCE NO
$CH_3\ CH_2\ CO\ COOH + O = CH_3\ CH_2\ COOH + CO_2$ (α-ketobutyrate) (propionate)	1
$\begin{matrix} CH_2\ COOH \\ \vert \\ CO\ COOH \end{matrix} + O + CH_3\ CO\ COOH = \begin{matrix} CH_2\ COOH \\ \vert\ OH \\ C \\ \vert\ COOH \\ CH_2\ COOH \end{matrix} + CO_2$ (oxaloacetate) (pyruvate) (citrate)	2 3
$COOH\ CH_2\ CH_2\ CO\ COOH + O = \begin{matrix} CH_2\ COOH \\ \vert \\ CH_2\ COOH \end{matrix} + CO_2$ (α-ketoglutarate) (succinate)	4, 5
$2CH_3\ CO\ COOH + O = \begin{matrix} CO\ CH_3 \\ \vert \\ CH_2\ COOH \end{matrix} + 2CO_2 + H_2O$ (pyruvate) (acetoacetate)	6
$CO_2 + CH_3\ CO\ COOH = COOH\ CH_2\ CO\ COOH$ (?) (pyruvate) (oxaloacetate)	7 8
$CH_3\ COOH + 2O = 2CO_2 + 2H_2O$ (acetate)	9

(1) LONG, C., and PETERS, R. A. (1939). *Biochem. J.* 33, 759.
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because the diagnosis of pellagra has not been considered Its occurrence in the mentally deranged can sometimes be attributed to food fads and a consequently unbalanced ration Conversely, a deficient intake may itself be the cause of the mental symptoms.



FIG 19. Pellagra in a mental patient.

Diagnosis of pellagra in the early stages may be helped by a history of dietary deficiency and in doubtful cases it may be useful also to make a chemical determination of nicotinic acid derivatives excreted in the urine after administration of the vitamin in a saturation test (cf p 62) In fully developed pellagra the diagnosis is easy being based on the characteristic skin lesions glossitis, stomatitis, diarrhoea mental symptoms and the response to a therapeutic test with the vitamin

Prognosis and Treatment

The treatment of pellagra was *revolutionized by the* identification (see below, p 57) of nicotinic acid (or amide) as the P P factor * The amide is preferably used for therapeutic purposes since unlike the acid it does not give rise to unpleasant side effects flushing and tingling in the skin when given doses In severe cases, doses of up to a total o of nicotinamide per day may b , o begin

* The amide is considered to be the a ... tioning in the body The acid, when ingest ... becomes the organism into the amide.

reduced later to 50–100 mg A dramatic improvement in the mental condition is commonly seen within a few

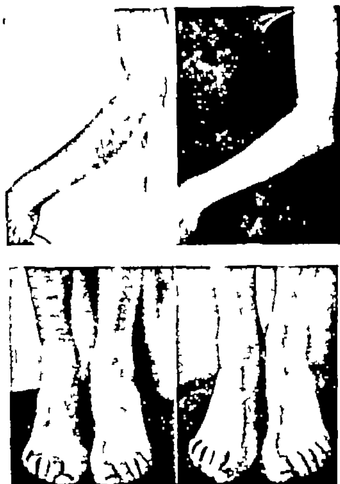


Fig 21 Pellagra, before and after treatment (left and right, respectively) (After Simpson)

hours of the first dose and in the lesions of the skin and the mouth within a few days. A daily allowance of 50 of nicotinamide is sufficient to prevent relapse At

of diet rich in nicotinamide—some of which should be supplied in preventive as well as in curative treatment—include those mentioned below i.e. yeast, liver (or yeast and liver extracts) canned salmon, meat, whole wheat, or the bran or germ of wheat or rice.

In conditioned deficiency caused by faulty absorption or utilization of the vitamin, parenteral administration of nicotinamide may be required

Concurrent Deficiencies

Since the diet of pellagrins is likely to be low in various associated factors as well as in the P P factor the provision of a satisfactory mixed diet for the correction of concurrent deficiencies is important For example the polyneuritic signs sometimes seen accompanying pellagra will need vitamin B_1 for their cure and lesions of the lips and face may respond to riboflavin, or possibly other constituents of the vitamin B_2 complex which in general occur together with nicotinic acid in the foodstuffs just mentioned

Daily Requirement

The daily requirement of *nicotinamide (preventive) is* thought to be of the order of 10–12 mg but it probably depends on other variables, e.g the amount of tryptophan in the diet, and perhaps on the extent of microsynthesis (p 60)

PELLAGRA AS A DIETARY DEFICIENCY DISEASE

Dietary Origin

Proof that pellagra was not conveyed by infection but was a deficiency disease was first furnished by Goldberger and his co-workers (1914 and 1915) In the first place he

solubility as follows —(1) Certain *wheat embryo extracts* contained relatively more of the antineuritic, and *liver extracts* more of the P P factor (2) Heat treatment in an autoclave destroyed the antineuritic, but not the P P activity (3) The antineuritic factor was more soluble, and the P P factor less soluble in strong alcohol

Experimental Pellagra

This advance—the differentiation of a separate P P vitamin—was made possible by the discovery by Gold berger *et al* of an experimental condition in dogs, black tongue, analogous with human pellagra. Since then pellagra has been produced in monkeys (Harris 1937 1938) and in pigs (Birch Chick and Martin 1937 Chick *et al* 1938) The disease in these animals is prevented



FIG 21 Experimental pellagra in the pig



Above—pig fed on diet of maize and casein.
Right—same pig after treatment with nicotinic acid.
(*After Chick Macrae, Martin and Martin* 1938)

by the same means as human pellagra Rats, on the other hand do not readily develop symptoms of disease when kept on a diet which will give rise to pellagra in man, dogs, monkeys, or pigs (cf below p 59) A condition accepted by Goldberger at the time (1926) as being rat pellagra was later shown to be due to deficiency of another factor vitamin B_6.

Nature of P P Factor

As already mentioned Birch, György and Harris in 1935 characterized the P P factor as a third component of the vitamin B_2 complex (the heat stable part of vitamin B) different both from this rat pellagra factor and from riboflavin (see p 17) In 1937 Elvehjem and his collaborators tested nicotinic acid upon dogs suffering from pellagra (black tongue) and found it to be active. It was equally effective in curing pellagra in monkeys (Harris) or pigs (Chick *et al*) and very soon afterwards was tried in human pellagra by three different groups of investigators (Fouts *et al* 1937 Harris and Hassan 1937 Spies *et al* 1937) and at once met with unqualified success

The earlier nutritional history of nicotinic acid is of much interest A quarter of a century previously (1912–1914) Funk in England and Suzuki in Japan had isolated nicotinic acid from concentrates of vitamin B Since at the time vitamin B was not yet recognized as a complex, it was natural to assume that nicotinic acid was related to the *anti neuritic* factor or to vitamin B_1 as we should now say Subsequent developments, however disproved this supposition and therefore the suggestion that nicotinic acid was a vitamin was almost forgotten

Much later in 1935 Euler and his collaborators on the one hand and also Warburg and Christian on the other found that nicotinic acid or rather its acid amide, was a

component of certain hydrogen transporting co-enzymes In 1937 Knight in England and Mueller in U.S.A proved nicotinamide to be a growth factor for certain

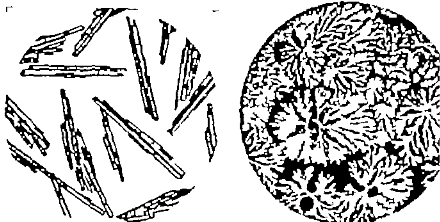
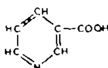


Fig 22 Crystals of nicotinamide (*left*) and nicotinic acid (*right*)
(*After Racke and Bucknell and Prescott*)

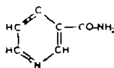
micro-organisms. Its identification as the P P factor followed also in 1937 as mentioned above.

Chemical Constitution of Nicotinic Acid and Nicotinamide

Nicotinic acid has the simple constitution of pyridine β-carboxylic acid and is biologically active, being converted into its acid amide, nicotinamide, on ingestion (p 52) Many other related substances have been found to be inactive



Nicotinic acid



Nicotinamide

Nicotinamide Deficiency in Rats

It was shown by Krehl and his colleagues (1945 and 1946) that the effects of a deficiency of nicotinamide could be demonstrated in young rats, although only by an impairment in their growth provided that appropriate measures were taken These were that the diet should be

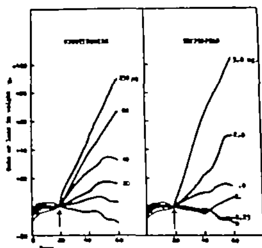


FIG 23 Nicotinamide deficiency in rats.

Failure in growth caused by the use of diet low in protein and containing maize Cured by the addition of nicotinic acid or nicotinamide—or of tryptophan, which acts as precursor of nicotinamide

The above chart shows the average growth curves of groups of rats receiving graded daily doses of nicotinamide and of tryptophan dosing begun at arrow

(*Harris and Kodicek 1950*)

concurrently deficient in protein especially in the amino-acid tryptophan and also that it should include certain pellagragenic ingredients such as a large proportion of maize In the view of some workers the maize is thought to contain some independent anti vitamin or pellagragenic agent An imbalance in the amino-acid content of the diet may also accentuate the deficiency in rats thus addition of threonine or phenylalanine and some

tests It is not yet clear however whether these observations may have any practical application in medicine and dietetics

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among the troops at the various fronts there were many thousands of cases During the Second World War deficiency of vitamin C was successfully prevented in Great Britain by measures such as the provision by the Government of supplies of concentrated orange juice or black currant juice or synthetic ascorbic acid for mothers and young children and by the encouragement of the cultivation of fresh fruits and vegetables in allotments

The disease is liable to occur sporadically in isolated communities or individuals cut off from supplies of fresh fruits and vegetables or among those on expeditions and campaigns It may be encountered in institutions where the dietary arrangements are faulty or among bachelors living alone and it is still liable to occur endemically in Polar regions during the winter months It is occasionally seen in the tropics among gangs of coolies or labourers who are fed on an unsuitable dietary Outbreaks are on record among native mine workers in S Africa

An occasional cause of the disorder as mentioned below (p 81) is a restricted diet given in the course of medical treatment.

The diagnosis of scurvy is sometimes missed because it is not looked for

Symptoms

Scurvy in adults may take from 4 to 8 months to develop *on a vitamin C-free diet—or of course less if the previous* diet had been low in the vitamin According to the classical literature on the subject, the early signs may include weakness and lassitude, and fatigue on exertion. The first pathological change generally *to* be detected, According to more modern investigation may be a

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vy h[illegible] follicles followed by peri

MUELLER, J H. (1937) *J* 120

and As the disease develops,

(1937) *J biol Chem.* 120
QUASTEL, J H (1946) *Proc. N* [illegible]

skin or the subcutaneous,

inter muscular or subperiosteal tissues either sp
or as the result of injury these may appe
petechiæ or ecchymoses, and the effusion
can sometimes give rise to large swellings
the gums begin to swell and become increasin



FIG. 5 Left Case of scurvy showing the petechial hæmorrhages in the skin (After Harvey.)



FIG. 26—Hæ gums, in pati scurvy (After

and enlarged the margins become ulcerated and th
breath fœtid and finally the teeth get loose and fall ou
Bronchitis and other infections are not unlikely to occu
as complications A susceptibility to cardiac lesions ha
also been stressed as has al the delayed healin
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In infantile scurvy the pic
will be indicated below (p

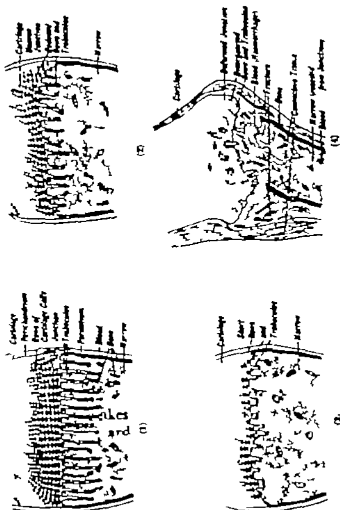


FIG. 29. Effect of scurvy on structure of bone.

(*After Dalf and Tozer*)

Diagram of rib junctions of guinea pig (1) Normal (2) Incipient scurvy (3) "Definite" scurvy (4) Acute scurvy

six to eight months, although it must clearly depend on the previous dietary history (cf p 68) Most cases occur between the ages of 6 and 18 months



FIG 30. Effect of vitamin C on growth of bone

(*After Ranke*)

Long bone of guinea pigs, showing impairment of osteogenesis in vitamin C deficiency Graded improvement with increasing adequacy of vitamin-C intake. I – no vitamin C in diet II – 0 25 mg per day III – 1 0 mg. per day IV – 5 0 mg. per day

Prognosis and Treatment

Scurvy responds dramatically to treatment with vitamin C and the prognosis is good unless complications have supervened An improvement can generally be detected in 24 to 48 hours and the cure may be complete within about 2 to 3 weeks For maximal rapidity of cure, large doses of ascorbic acid may be given for about a week say 750 mg daily (for an adult) and thereafter about 100 mg a day until recovery is complete. Alternatively lemon orange, grapefruit or black currant juice may be used Local treatment for the gums is advisable.

For an infant, a dose of 20–40 mg to begin with, has been found adequate For prophylaxis one orange a day for an adult or its vitamin C equivalent in the form of other fruits or synthetic ascorbic acid, is ample (cf above) An infant receives about 30 to 50 mg of ascorbic acid daily in average human milk (Harris and Ray 1935) and this figure has been used as a basis for calculation of normal needs

Vitamin C Therapy in Absence of Scurvy

Treatment with vitamin C has been recommended for

a number of hæmorrhagic conditions other than scurvy but there is no evidence that it is effective. In infectious diseases, however the need for the vitamin is increased and special provision of it should be made (see below pp 78–79)

VITAMIN C INTAKE REQUIREMENTS AND ASSESSMENT OF STATUS

Assessment of Vitamin C Status

The urinary saturation (or loading) test involves the measurement of the amount of vitamin C excreted in the urine after a series of daily test-doses The greater the deficit in the past intake, the smaller will be the excretion and the greater the number of daily test-doses needed to bring about a state of saturation i e. to give rise to a continuous large overflow into the urine. It has been shown that subjects who are suffering from scurvy or are about to develop scurvy take from seven to ten days of standard test dosing* before becoming saturated subjects whose past intake has averaged about 30 mg per day (League of Nations standard) take one or two days only while those who have been receiving 50 100 mg (U.S.A. so-called optimum standard) show the full response almost with the first test dose. With various intermediate intakes the responses are graded in proportion (The standard test dose is 700 mg per 10 stone of body weight.) These observations enable a reliable estimate to be made of the past intake from the number of days before saturation is reached (Harris 1942 1943 Nutrition Society 1945)

* Representing total intake of 7 g of ascorbic acid in the test doses. For earlier literature regarding development of test and details of technique see also Harris and Ray 935 ; Abbasy Harris, Ray and Marrack, 935 Abbasy Harris and Hill, 937 Harris and Abbasy 937

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* Representing total intake of 7 to 1 g of ascorbic acid in the test doses. For earlier literature regarding development of test and details of technique see also Harris and Ray 1935 Abbasy Harris, Ray and Marrack, 1935 Abbasy Harris and Hill 1937 Harris and Abbasy 1937

six to eight months, although it must clearly depend on the previous dietary history (cf p 68) Most cases occur between the ages of 6 and 18 months



FIG 30. Effect of vitamin C on growth of bone.

(After Reche)

Long bones of guinea pigs, showing impairment of osteogenesis in vitamin C deficiency Graded improvement with increasing adequacy of vitamin-C intake. I = no vitamin C in diet. II = 0 25 mg. per day III = 1 0 mg. per day IV = 5 0 mg. per day

Prognosis and Treatment

Scurvy responds dramatically to treatment with vitamin C and the prognosis is good unless complications have supervened An improvement can generally be detected in 24 to 48 hours, and the cure may be complete within about 2 to 3 weeks For maximal rapidity of cure large doses of ascorbic acid may be given for about a week, say 750 mg daily (for an adult) and thereafter about 100 mg a day until recovery is complete. Alternatively lemon orange grapefruit or black currant juice may be used Local treatment for the gums is advisable.

For an infant, a dose of 20–40 mg., to begin with has been found adequate. For prophylaxis one orange a day for an adult, or its vitamin C equivalent in the form of other fruits or synthetic ascorbic acid is ample (cf. above) An infant receives about 30 to 50 mg of ascorbic acid daily in average human milk (Harris and Ray 1935) and this figure has been used as a basis for calculation of normal needs.

Vitamin C Therapy in Absence of Scurvy

Treatment with vitamin C has been recommended for

a number of hæmorrhagic conditions other than scurvy but there is no evidence that it is effective. In infectious diseases, however the need for the vitamin is increased and special provision of it should be made (see below pp 78–79)

VITAMIN C INTAKE REQUIREMENTS AND ASSESSMENT OF STATUS

Assessment of Vitamin C Status

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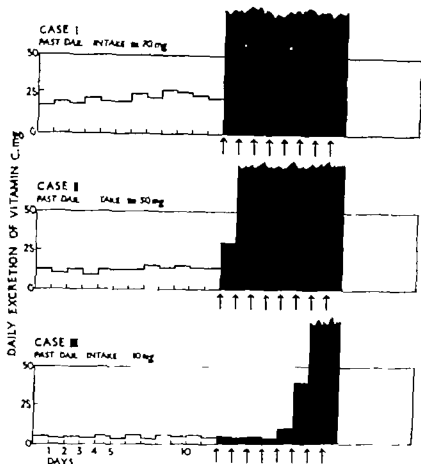


FIG 31 Principle of saturation test, for assessing status in vitamin C.

The lower the past daily intake of the vitamin, the less "saturated" will be the tissues of the subject, and in consequence the greater the number of days of test-dosing necessary (shown by arrows) before a large excess begins to overflow into the urine

Thus, with a past daily intake of about 70 mg the patient becomes saturated on the first day of test dosing, with an intake of about 30 mg one to two days' test dosing will be needed, with an intake of less than 10 mg upward of four days may be needed Patients with an extreme degree of deficiency i.e. suffering from scurvy will take about seven to ten days to become saturated.

Other Methods

An estimate of the level of nutrition can also be gained from tests on blood* or cerebrospinal fluid but the procedures are less simple and have not come into such wide use as the urinary tests

Tests of capillary fragility have not fulfilled the early expectation of forming a useful method for assessing status in vitamin C

Daily Requirement of Vitamin C

The physiological minimum for an adult is thought to be in the region of about 15-20 mg of ascorbic acid per day or a little less This figure agrees with calculations based on the amount of lemon juice which had been found to be necessary to counteract incipient symptoms of scurvy according to some old observations in the Navy Furthermore in some experiments on a group of human volunteers, carried out in 1944-6 it was observed that a dose of 10 mg was sufficient to cure or to prevent symptoms of clinical scurvy some doubt remained however whether this was the *optimum* dose needed for maximum efficiency under conditions of stress (Medical Research Council 1948)

The League of Nations standard requirement (30 mg) thus seems to allow a reasonable margin for safety and for human variation

Special Needs

With hard physical effort the need for the vitamin is increased as it is also during pregnancy and lactation In fever again there is an increased demand (see below pp 78-79) Children probably require about twice as much as adults per unit of body weight

* E.g. by Roe method see p. 86

Hypovitaminosis C Sub scurvy Latent scurvy

For many years it was maintained by some pædiatricians that incipient, latent, or subacute scurvy in infants was relatively common but often escaped diagnosis, or was mistaken for rheumatism (Hess, 1916 1920 Comby 1921) A difficulty was that the symptoms were described as vague the child was sallow and fretful but not wasted Provision of additional antiscorbutic food was said to lead to improved stamina and growth and to a lessened irritability Similar suggestions were made concerning a condition of sub-scurvy in adults (Stefánsson 1918 Sherman 1927 Abs 1928) and also in experimental animals (Meyer and McCormick, 1928)

Much of this is still controversial but on the whole the balance of evidence seems to suggest that it is possible for an individual on a diet poor in vitamin C to remain free from any obvious symptom of scurvy and yet to be so near a state of deficiency that it only becomes apparent when for example, wounds fail to heal satisfactorily after surgical operations, or ulcers bleed With such partial deficiency' of vitamin C there may also very well be an increased liability to infection and with children a delay in growth. The level of nutrition in vitamin C can be assessed by the saturation test mentioned above.

Vitamin C and Infection

Application of the 'loading test (p 75) has shown for any given intake of vitamin C, the excretion is sed in infectious diseases notably so in pulmonary osis (Abbasy Harris and Ellman 1937) This hat there is an increased usage of vitamin C in conclusion confirmed by actual analysis of the tissues of infected guinea pigs (Harris, Pagel 193,) One effect of deficiency of

vitamin C certainly in guinea pigs is a susceptibility to infection There is also a impression that the same is true of human b role of vitamin C in infection is, however stil (for discussion see Harris, 1937)

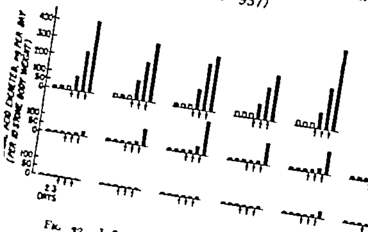


Fig 32 Influence of infection in lowering the vitamin-C status.

(After Abbas Harris and Ellman, 1937)

Various infective conditions, notably phthisis, cause an increased wastage of vitamin C in the body and hence an increased requirement in the diet

Six cases of "active" pulmonary tuberculosis (bottom line, in chart above) gave evidence of a very low state of saturation by excreting little vitamin in their urine, and giving little or no response after three successive doses. In comparison, six normal controls, on quiescent cases, on same diet all gave good responses. Six cases of "moderate" severity gave intermediate responses

...ive of Vitamin C

...been drawn to the marked seasonal ... the vitamin. Thus as much as 50–60 ...ed in a day in the summer months in a ...arding school for boys where good use was home-grown garden products, whereas in the

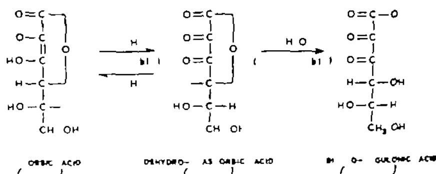
Instability

From a practical point of view one of the most important properties of ascorbic acid is its great instability due to its liability to destruction by oxidation This inactivation is greatly accelerated by heat and alkali and by the presence of copper which acts as a catalyst.

An enzyme, ascorbic oxidase present in various plant tissues also promotes the inactivation of ascorbic acid it is referred to again below (pp 86 89)

Dehydroascorbic Acid

The first product formed in the oxidation of ascorbic acid is dehydroascorbic acid This is still biologically active. Under appropriate conditions, this stage of the oxidation can be reversed The dehydroascorbic acid like ascorbic acid itself is very unstable and with further oxidation the activity is lost irreversibly



Oxidation products of vitamin C.

Dehydroascorbic acid can be prepared in the laboratory by mild oxidation of ascorbic acid e g by a process involving adsorption on charcoal Treatment with H_2S regenerates the ascorbic acid

Other Chemical and Physical Properties

Unlike vitamin B_1, vitamin C cannot be adsorbed on fullers earth and similar reagents. It dissolves readily in water and methyl or ethyl alcohol and in various aqueous media, but not in fat-solvents.

METHODS OF ASSAY

Biological Methods

The standard method of estimating vitamin C in food stuffs was for a long time that introduced by Holst namely a determination of the minimal dose needed to prevent typical signs of scurvy in guinea pigs A simpler alternative to this classical preventive test with guinea pigs is a semi-curative growth test (see Harris and Olliver 1942)

Another sensitive test is based on the microscopical examination of tooth structure (Höjer 1924 1926 Key and Elphick, 1931)

Chemical Methods

Tillmans (p 82) observed that many active foodstuffs reduced the dye-stuff 2 6-dichlorophenolindophenol and, by the introduction of a preliminary extraction process and by carrying out the titration rapidly in acid solution Harris and Ray (1933 1) made this the basis for a more specific quantitative method of chemical estimation (see Birch, Harris and Ray 1933 Harris, 1937 2) This has been shown to give reliable results and to agree with biological assays for ordinary food stuffs, such as numerous raw or cooked fruits and vegetables (Harris and Olliver 1942) The only important exceptions known to the specificity of this simple method of titration applied to common foods and tissues are (a) with products which have been caramelized

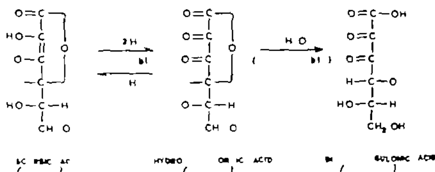
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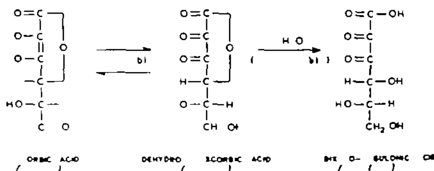
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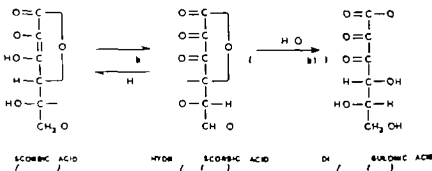
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(darkened or charred) as the result of the application of high temperature treatment (for example, some dehydrated foods or roast potatoes) (b) with certain fermented foods and (c) with urine and for these, special modifications in procedure must be used (see below)

With the caramelized products mentioned above, containing interfering substances (gluco-reductones) the ascorbic acid can be quantitatively differentiated from the interfering substances by (1) measuring the actual velocity of the reaction with the indophenol reagent (Harris and Mapson 1944 1947) or (2) comparing the rates of condensation with formaldehyde (Mapson, 1943)

Sulphite (SO_2) sometimes added to foodstuffs as a preservative will also interfere by reducing the dye, and must be removed before the titration either by the addition of acetone or by being blown off

In [illegible] with deeply pigmented solutions a potentiometric method is available (Harris, Mapson and Wang,

[illegible] chemical method depends on the use of dinitrophenylhydrazine (Roe and Kuether applied by Lowry, Lopez and Bessey 1945 to blood plasma) The polarograph (polarizable dropping mercury electrode) has also been employed (Kodicek and Wenig 1938)

Alleged Bound Ascorbic Acid Dehydroascorbic Acid

The supposition that a bound form of ascorbic acid existed in raw foods and that it was liberated on cooking, seems to have been based on a misapprehension, the explanation being that the heat of cooking destroys an [illegible] present in the raw food this oxidase inactivates the vitamin during the actual course of the extraction [illegible] the raw food if adequate precautions were not taken to prevent it Nor has the view been accepted that

appreciable amounts of the reversibly-oxidised form of the vitamin dehydroascorbic acid are present in ordinary foodstuffs or processed materials

Distribution in Foods

The best natural sources of the vitamin include various fresh fruits and vegetables. Figures cited below in brackets are typical ranges of values for the ascorbic acid content in mg per 100 g *Fresh fruits* —Orange juice (50–70) lemon juice (40–60) grapefruit (35–45) tomato juice (20–30) pineapple juice (30) strawberries (60–80) black currants (200) Other fruits such as apples, cherries greengages, pears, plums, contain much less (3–5) Many *fresh green vegetables* are highly active, including kale (120–140) Brussels sprouts (90–110) cauliflower (60–80) cabbage (60–80) spinach (60–70) and green leaves generally Other potent vegetable products include fresh green peas (20–30) turnips (20–40) and new potatoes (30–40) Poorer is lettuce (12–15) and relatively inactive are celery marrow radishes (5–7) and mush rooms (0)

It was Szent-Györgyi who in 1933 discovered that **paprika** is exceptionally rich in vitamin C, and Olliver (1936) who showed the same to be true of **black currants** (values of about 200 mg per 100 g) Other notable figures are papaya, 50–100 and guava 50–100 Probably the most active known source is **rose hips** (1 000) and since 1942 a rose hip syrup (175–200) has been issued by the British Ministry of Food

Fresh meat contains small but appreciable quantities of the vitamin.

Most ageing and drying processes entail a consider able or complete loss of activity Thus fruits and vegetables as purchased at the market may as a result of wilting be appreciably poorer than those freshly gathered

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Canned fruits and vegetables are found to vary considerably in their activity, canned tomatoes providing an example of a good antiscorbutic foodstuff. Orange marmalade, and some jams (e.g. black currant, strawberry) may also contain useful amounts of the vitamin.

In commercial pasteurization of milk, the presence of copper in the containers has been responsible for the destruction of the vitamin. A further loss occurs when milk is left in bottles exposed to the light, the photocatalytic destruction being sensitized by riboflavin (Kon and Watson, 1936; Hopkins, 1938).

The vitamin C in the plant organism first appears when the seed germinates (Fürst, 1912), and this principle has sometimes been put to good practical use, as when a supply of **freshly sprouted grain** has been used to furnish an antiscorbutic pabulum on expeditions of exploration and military campaigns.

It is worthy of note that certain foods which are rich in vitamin B_1—including yeast, yolk of egg, and cereals—are distinguished by being **entirely devoid of vitamin C**.

For full lists of ascorbic acid contents of foods see Fixsen and Roscoe (1938, etc.) and the later British Tables (e.g. Nutritive Value of Wartime Foods, 1945; Olliver, 1943) or the American counterparts (Tables of Food Composition, 1945).

Losses on Cooking and Processing

Some loss of vitamin C during cooking of vegetable foods is almost unavoidable. The loss is due partly to oxidation of the vitamin and partly to its dissolving into the water used for the cooking. Such losses can be minimized (Olliver, 1941; Allen and Mapson, 1944) if care is taken.

(1) to avoid undue crushing or chopping of the vegetable into too small pieces (whereby the oxidizing enzymes are set free)
(2) to have the minimum of water
(3) to start with the water boiling instead of bringing it to the boil (to cut short any oxidative processes)
(4) not to keep the cooked vegetable on the hot plate for longer than necessary (mashed potatoes rapidly losing almost all their vitamin on being kept hot)

In the cooking of acid fruits, e g gooseberries it is safe to add bicarbonate of soda in order to save sugar—provided that an excess is avoided which would spoil the colour and the flavour as well as the vitamin value (Mapson and Barker 1941)

Dehydrated Foods

During the Second World War it was found a great convenience for the fighting Services to have a readily available supply of vegetables in the dehydrated form Being less bulky they were easy to transport, and could be used in regions, e.g in the desert campaign, where fresh vegetables and facilities for cooking them were not forthcoming Other advantages of the use of dehydrated vegetables are (1) that seasonal gluts can be saved and used subsequently and (2) that choice products can be sent to distant lands where they are not normally grown Investigations at the Low Temperature Research Station and the Dunn Nutritional Laboratory Cambridge and in the U.S.A and elsewhere resulted in the evolution of a manufacturing procedure by which vegetables could be dried without serious loss of their vitamin value The process includes (1) a preliminary scald with sulphur dioxide (2) drying under specified conditions of temperature humidity etc and (3) storage in an inert gas. (For

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In the cooking of acid fruits, e.g gooseberries it is safe to add bicarbonate of soda in order to save sugar—provided that an excess is avoided which would spoil the colour and the flavour as well as the vitamin value (Mapson and Barker 1941)

Dehydrated Foods

During the Second World War it was found a great convenience for the fighting Services to have a readily available supply of vegetables in the dehydrated form Being less bulky they were easy to transport, and could be used in regions, e.g in the desert campaign where fresh vegetables and facilities for cooking them were not forthcoming Other advantages of the use of dehydrated vegetables are (1) that seasonal gluts can be saved and used subsequently and (2) that choice products can be sent to distant lands where they are not normally grown Investigations at the Low Temperature Research Station and the Dunn Nutritional Laboratory Cambridge and in the U.S.A. and elsewhere, resulted in the evolution of a manufacturing procedure by which vegetables could be dried without serious loss of their vitamin value. The process includes (1) a preliminary scald with sulphur dioxide (2) drying under specified conditions of temperature, humidity etc and (3) storage in an inert gas. (For

further details see *Proc Nutrl Soc* 1942, or for a review of American work 'Vegetable and Fruit Dehydration (1944))

PHYSIOLOGICAL ACTION

Biochemical Role

It is supposed that the action of vitamin C must be linked in some way with its intense reducing action which is associated with the presence of the characteristic **ene diol** grouping $-C(OH){=}C(OH)-$ It has to be admitted, however that the exact biochemical role of vitamin C is still obscure, although there is probably significance in the recent findings that in animal tissues it can catalyse the oxidation of the side chain of the aromatic amino-acid tyrosine (Lan and Sealock, 1944 Darby *et al* 1945) There are suggestions that in the plant kingdom vitamin C may be concerned in the catalytic oxidation of lactate to pyruvate (James and Cragg 1943) and in the triosephosphate system (James, Heard and James 1944) as well as the tyramine-quinone equilibrium (Miller and his colleagues, 1944)

Morphological Changes

It was shown by Wolbach and Howe, in 1926, that vitamin C is needed for the production of collagen, or intercellular cementing substances. Thus absence of collagen is one effect of deficiency of vitamin C.

Furthermore as was demonstrated by Fish and Harris (1934) the formative cells of the body such as the osteoblasts, odontoblasts and ameloblasts, lose their normal functional activity in the absence of vitamin C thus the manifestations of scurvy may be regarded as due to an impairment in the function of such formative cells.

Diagrammatical representation of longitudinal section of the incisor tooth of guinea pig (growing continuously in direction of arrow) to show the effect of three successive diets in turn

(1) *Normal.* With adequate vitamin C the odontoblasts or dentine forming cells (O) are arranged in orderly columns around the pulp chamber (P) and produce regular tubular dentine (D).

(2) *Partial deficiency.* The odontoblasts become partly degenerated and are no longer able to produce normal tubular dentine. Some irregular dentine is formed and invades the pulp ca[illegible]

(3) *Severe deficiency.* Odontoblasts completely degenerated. Little or no new dentine is formed [illegible] enamel (E) and cementum [illegible] are [illegible]

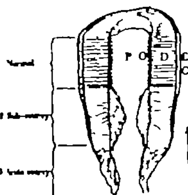


Fig. 35. Effect of lack of vitamin C on tooth structure.
(*After Harris*, 1935)

Explanation of Hypovitaminosis C

The foregoing theory seems to account for the remarkable changes seen in the teeth of experimental animals suffering from a slight or partial deficiency of vitamin C (Fish and Harris, 1934) for the no less remarkable overgrowth of bony tissue observed in some circumstances (Kodicek and Murray, 1943, etc.), and no doubt also for the poor healing of wounds in scurvy or in the subscorbutic state. The probable significance of these findings in human nutrition still remains to be worked out.

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CHAPTER VI

VITAMIN D AND RICKETS

Rickets is a disease of nutrition seen generally (b
not exclusively) in early childhood It is caused by la
of vitamin D and the most characteristic signs are t
deformities of the growing bones

RICKETS IN INFANTS

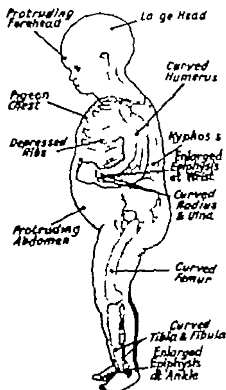


Fig 56. Diagram of deformities in rickets.

(Reproduced from Harris, "Vitamins in Theory and Practice" 1935, after Hermann et al.)

The Conquest Rickets

At the close of t
19th century and t
beginning of the 20
cases of severe ricket
easily recognized by t
well known bo
changes, such as kno
knees or bow legs p
eon chest and beadi
of the rib junctio
curved spine rach
pelvis and bossing
the forehead —were s
very common in t
British Isles Inde
in some northern
dustrial areas rick
was so prevalent as to
almost universal T
eradication of sev
rickets is one of t

major triumphs of modern medical research but although no child need have rickets mild rickets still occurs





FIG. 57 Cases of severe rickets. Top left, in negro children right, white child

(After Mayer and F. McGrath)

Incidence in the Past

In 1868 [severe] rickets was said by Gee to have occurred in about 33 per cent of London school children and in 1871 the same figure was given for Manchester by Ritchie * Between 1915 and 1928 official reports concerning school children from the L.C.C. and Durham gave evidence of some degree of rickets in 80 to 87 per cent A survey by the Ministry of Health in 1926 gave a

* The more modern methods by which the milder manifestations of rickets can be diagnosed were of course at that time unknown

50 per cent incidence among 1 000 children examined throughout England and Wales

Continued Prevalence of Mild Rickets

With the growth of knowledge and the spread of the infant welfare movement rickets is becoming less common *and the very severe cases once so familiar* are now comparatively rare. But it has been emphasized that after *the winter months* and in the absence of preventive treatment, some degree of rickets in a proportion of infants *is almost inevitable in such climates as those of Britain or* many parts of Northern Europe and North America (Gebhart, 1924 J H *Hess et al* 1930) *Preventive* treatment is often neglected and surveys, in which the modern more sensitive methods of diagnosis were used have in fact indicated that the incidence of mild rickets was still surprisingly high (Newman 1929 Eliot 1925, 1926 Crawford and Williamson 1930)

A national survey was carried out by the British Pædiatric Association at the suggestion of the Ministry of Health in 1944, to ascertain whether there had been any increase of rickets in war time In 23 areas in Great Britain and Ireland a total of 5 283 children between the ages of 3 months and 18 months were examined both for the presence of clinical signs and for radiological evidence of rickets The conclusion reached was that the incidence of rickets diagnosed radiologically in children between 3 and 18 months of age is 2½ per cent before 6 months, *4 per cent in the first year* and negligible after that period This meant that about 24 000 children in Great Britain suffered from rickets every year The rates for active rickets diagnosed **clinically** (enlarged epiphyses, enlarged costochondral junctions delay in closure of the fontanelle craniotabes late walking and delayed

dentition) ranged from nil in St Albans and Watford to 64 per cent in Sheffield *

Early History

Most medical historians have overlooked the fact that in 1645 a young medical student of 26 years of age, named Daniel Whistler (hailing from Merton College, Oxford) published in Leyden a Thesis for his M D degree entitled *De morbo puerili Anglorum quem patrio idiomate indigenae vocant* The Rickets This is probably the first known account of rickets and antedates the more celebrated book of the Cambridge clinician Glisson *De Rachitide* published in 1650 On the continent of Europe, rickets was long known as *die englische Krankheit* in allusion either to the writings of Glisson and other English authors or to its common occurrence in England



Fig 38 Title page of Glisson's treatise on rickets published in 1650.

From 1782 onward the

* The report again stresses the finding that although in severe cases the clinical diagnosis of rickets is usually easy, in the milder forms it is extremely difficult, and in the minor degrees of bone change quite impossible For the latter radiological diagnosis is desirable A chemical analysis of the blood serum, for inorganic phosphate and calcium, or still better for phosphatase (alkaline phosphatase) is the most sensitive test for incipient rickets (*see below* p. 104)

use of cod liver oil for rickets gradually gained ground and by 1865 it was confidently recommended by the great French clinician Trousseau as the well known and perfect cure for rickets Already in 1838 Jules Guerin had produced rickets experimentally in puppies to substantiate the theory that it was a disease of faulty diet, and the English surgeon Bland Sutton, some 50 years later had cured rickets in lion cubs at the London zoo by the use of cod liver oil together with milk and crushed bones. Nevertheless clinical teaching remained confused and divided and it was not until 1918-1923 that the ætiology of rickets as a deficiency disease became clearly established.

Recognition of the Antirachitic Vitamin

As mentioned on p 12 Hopkins in 1906 alluded to rickets as a disease. in which for long years we have had knowledge of a dietetic factor' and in 1912 Funk postulated that rickets was one of the vitamine deficiency diseases. Conclusive proof of the existence of an anti rickets vitamin was first given when Mellanby (1918) showed that experimental rickets in dogs could be prevented by the inclusion of certain fats in their diet, such as cod liver oil or butter but not by various vegetable oils such as linseed or olive oil Soon afterwards followed the production of rickets in rats (McCollum *et al* 1921 Sherm[illegible] and Pappenheimer 1921) and the diff[illegible] anti rickets vitamin—then to be [illegible] —from vitamin A (McCollum

[illegible] t

P[illegible] gnized (Palm 1890) that rickets
Brit[illegible] temperate *zone or* wherever the
active [illegible] in humid climates, in smoky or
enlarged[illegible] is [illegible] [illegible]ded industrial areas, or where the rite of *purdah*
the fontan[illegible]

bone at the epiphysis where the zone of provisional calcification is increased in breadth and inadequately mineralized An irregular growth of the soft osteoid

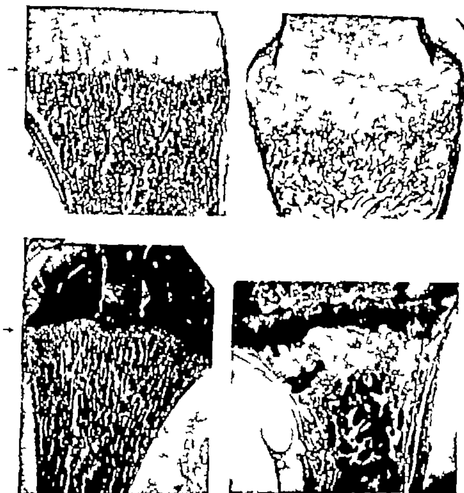


FIG 40. Effect of vitamin-D deficiency on bone structure
To left, in each case, normal bone to right, in each case, rachitic bone for comparison.

(*After Boyd and Aske, respectively*)

Longitudinal sections of epiphyseal end of long bone
In place of the straight, regular line of ossification in the normal bone (marked by arrow), there is seen in rickets, a wide irregular band of imperfectly calcified cartilage and osteoid tissue The end of the bone is abnormally widened

tissue follows and a consequent swelling of the epiphyseal junction (rachitic metaphysis) The bending of the inadequately calcified bones and the overgrowth of osteoid tissue cause the well recognized deformities already referred to—bow legs, knock knees rachitic pelvis, green stick fractures, the enlargement of the ends of the bones, and the beading of the ribs. Together

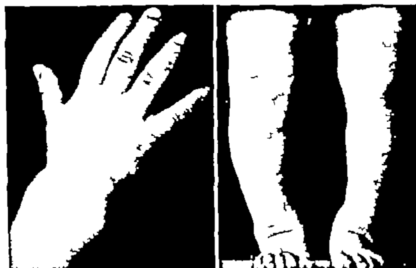


Fig 4 Enlargement and deformity of epiphyses at wrist and ankles in rickets

([illegible])

with the bony changes there is loss of tone of the muscles and the pot belly is a noticeable feature Tetany (p 106) may be present

Pathogenesis

The immediate cause of the deficient calcification of the bone in rickets is thought to be the low level of the inorganic phosphate in the blood plasma or more

correctly the low Ca × P product (Iversen and Lenstrup 1919 Howland and Kramer 1921 1922 1923) Ultimately this chemical defect in the blood is related to an inadequate net absorption (see p 118) of the P and/or Ca from the intestine. A low plasma P (or Ca × P product) is characteristic of active rickets

Laboratory Diagnosis

Use is made of the last mentioned fact for diagnosis Indeed an analysis of the blood serum either for P and



FIG 42 Radiological diagnosis of active and healing rickets. X-ray picture of bones of wrist (distal end of radius)

On left, *active rickets*, showing, at *A* the "rachitic metaphysis" i.e. lack of calcification of newly-forming bone at the growing end of the diaphysis. On right, *healing rickets*, showing, at *B* the recalcification in progress.

(*After Huldschinsky*)

Ca or for phosphatase (see Smith 1933) is the only certain method of detecting incipient rickets or mild rickets. The radiographic abnormalities at the epiphyses do not become apparent until a later stage, but they form the most useful guide in following the course of healing (cf footnote, p 97)

Prophylaxis and Treatment

If rickets is to be prevented with any degree of certainty in northern climates in the winter at least the infant's

diet must be supplemented with vitamin D This may be either in the form of cod liver oil or better halibut liver oil or concentrates of vitamin D_2 (calciferol) or D_3 Perhaps the most convenient procedure for artificially fed infants is to employ an infant food (e.g a proprietary dried milk preparation) in which a standardized amount of vitamin D has been incorporated Success has also attended the use of irradiated milk, or of helio- or actinotherapy but these measures are not generally so convenient.

In curative treatment halibut liver oil and calciferol have the advantage over cod liver oil that they can be tolerated at a more adequate level of antirachitic activity The optimal prophylactic dose for infants and children has been found to be from 500 to 1 500 i u daily and the optimal curative dose about 1 000 to 3 000 The minimal toxic dose may on occasions, be as low as 10 000 i u daily although very much more has often been given with apparent impunity—as in the so-called bomb therapy which has had its advocates. It nevertheless seems desirable that the more moderate levels of dosage above advocated should be adhered to the lower possible limit of toxicity being not too far removed from the optimal level for most effective curative treatment (see Harris 1933)

Vitamin D and the National Diet

During the Second World War the Ministry of Food in the United Kingdom arranged for all the margarine to be reinforced with vitamin D_2, and made available supplies of cheap or free cod-liver oil reinforced with vitamin D_2 for pregnant and nursing mothers and for children. A war time social survey however revealed the disconcerting fact that only 40 per cent of mothers

were collecting the cod liver oil to which they were entitled

Rickets and Dental Caries

It has been known since the last century at least, that rickets is often, although not invariably associated with dental caries, and it is now appreciated that vitamin D by promoting the normal calcification of the teeth, aids them to resist erosion Clinical trials have proved that administration of vitamin D is one of the factors which can help to keep in check the spread of caries (M Mellanby 1934) See further on p 122

VITAMIN D FOR THE MOTHER

In Pregnancy and Lactation

In prescribing vitamin D for the child, the need of nursing and expectant mothers should not be overlooked An allowance of about 1 000 to 2 000 i u. daily may be recommended The extra vitamin is needed not so much for any effect that it may have on the offspring or on the composition of the milk—which is small in ordinary circumstances—but rather in order to increase the mother's assimilation of calcium and phosphate, so as to make good that lost to the fœtus or in the milk.

INFANTILE TETANY CŒLIAC RICKETS JUVENILE RICKETS OSTEOMALACIA RENAL RICKETS

Tetany Associated with Rickets

Infantile tetany seen in association with rickets is a nutritional disorder due directly to the *low level of* calcium in the blood plasma. It is cured and prevented by the same measures as those which are effective in the

treatment of rickets. The view which used formerly to be propounded that tetany was due to malfunction of the parathyroid gland or to intoxication with guani dine certainly does not apply to true infantile (nutri tional) tetany In fact treatment by parathyroid hormone, which had sometimes been recommended inadvisable except as an emergency measure for it merel

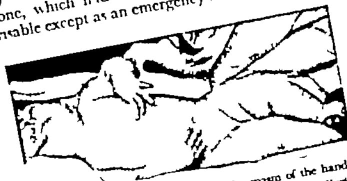


FIG 43 Child with tetany Note the spasm of the hand (After Hec

aggravates the underlying metabolic error caus of calcium from the body by taking it from the b

Cœliac Rickets

The failure in cœliac disease to assimilate fat those fatty foods which serve as carriers of v (cod liver oil etc) may lead to rickets. This tion can be prevented by the administration of in the form of concentrated synthetic prepa calciferol in the absence of any excess of f 1927 1931)

Late (Juvenile) Rickets

In western countries, adolescent or juv rare in contrast with infantile or true ricket mentioned the latter in its active form

always between the age limits of about six months to two years In Central Europe at the close of the First World War however juvenile rickets was prevalent (Simon

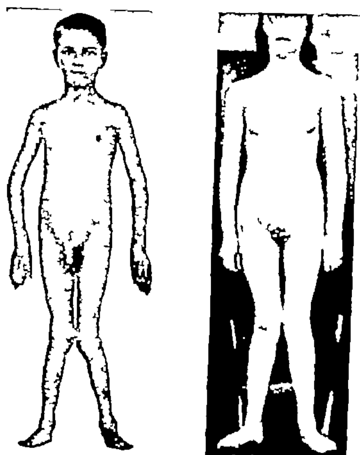


Fig 44. Cases of adolescent rickets.
N.B. knock knees and enlarged epiphyses at wrists, and ankles.
(After Ferr and Findlay)

1921) In recent decades, it has still been seen in a number of eastern countries including India (see e g Hutchison and Stapleton 1924 Wilson 1931) and elsewhere for example, among Finns and Laplanders (Kloster 1931)

Osteomalacia

Osteomalacia or adult rickets has been prevalent in a number of eastern countries, e.g. in Northern China (Maxwell and Miles, 1925) and in parts of India



Fig. 46. Narrowing of pelvis in osteomalacia, making normal childbirth impossible

([illegible] Royal College of Surgeons, London)

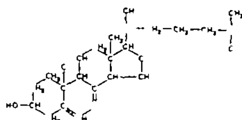


Fig. 4[illegible]. A severe case of adult rickets (osteomalacia)

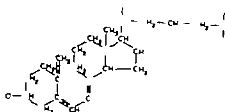
(After [illegible])

(Vaughan 1929; Wilson and Surie 1930). Its cause commonly lies in a combination of circumstances: there may be a lack of vitamin D from the diet or diminished exposure to sunlight, as when *purdah* (p. 98) is observed, *but often there is also an actual shortage of calcium or* phosphate in the diet. In Vienna during the food shortage after the First World War osteomalacia was prevalent in the late winter and spring among poor

Probably vitamin D_2 (calciferol) occurs naturally in irradiated vegetable tissues and vitamin D_3 is the principal form in most fish liver oils



7 Dehydrocholesterol (provitamin D_3)



Vitamin D_3.



FIG. 48. Rickets in chicks.

The chick on the right received no vitamin D. That on the left was given an adequate dose of vitamin D_3, and that in the middle the same amount by weight of vitamin D_2 (less effective than D_3 for [illegible]

(After MacLennan [illegible] Bri[illegible] Oil Produ[illegible]

Other D Vitamins

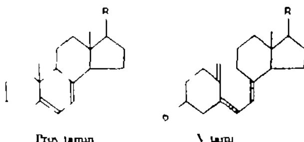
There are certain other variants of vitamin D in addition to D_2 and D_3, such as artificially prepared *irradiated 22 dihydro-ergosterol* (*vitamin D_4*) and *irradiated 7-dehydrositosterol* (*vitamin D_5*) but none of these seem of much practical importance.

Also as indicated above, fish liver oils may contain varying amounts of additional D-vitamins, distinct from the principal form D_3 but these have not yet been well characterized

Cholesterol under certain conditions of chemical treatment may also be made to yield a product possessing distinct antirachitic properties, but this differs considerably in properties and structure from the group of D vitamins as understood above

The Vitamin D Skeleton

The general skeleton formulae for all forms of the vitamin and provitamin are shown below R representing a variable side chain



Provitamin Vitamin
General formulae for the D provitamins and the D vitamins.

ASSAY OF VITAMIN D IN FOODS AND TISSUES

Alternative Assay Techniques

For the assay of vitamin D various alternative procedures are available The most satisfactory are the following

(i) curative tests on rats with X ray examination of the rachitic metaphyses (see Bourdillon *et al* 1931) (ii) the line test (McCollum *et al* 1922) similar to the foregoing

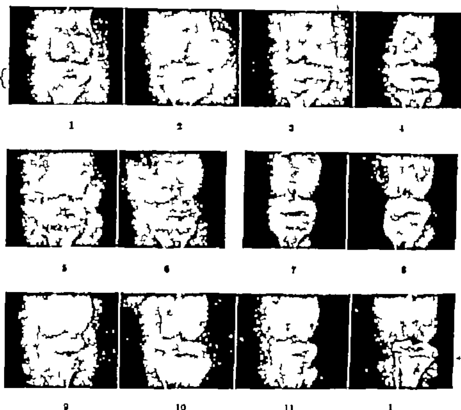


FIG 49. Radiological assay of vitamin D

In this radiological method for the assay of vitamin D the criterion used is the degree of healing produced in the bones of rachitic rats. In the X-ray pictures above are depicted twelve degrees of healing, corresponding with the increasing graded doses of vitamin D administered.

The appearance of the bone at the position marked by the arrow should be compared with increased healing the width of the uncalcified gap is diminished.

(After Bourdillon et al 1931)

but involving visual inspection of a silver stained preparation of the metaphyses *post mortem* and (iii) determination of the ash content of the bones Nos (i) and (iii) are

causes, directly or indirectly either an *increased absorption* of these elements from the intestine or alternatively their *diminished re-excretion* into the alimentary tract As it was not certain which of the two processes was more important, the action was expressed by saying that it caused an increased net absorption (Harris, 1932) This increased net absorption is reflected in a raised concentration of phosphate or calcium or both in the blood-stream (Iversen and Lenstrup 1919 Howland and Kramer 1921) This in turn promotes an increased calcification of the bone in other words the *defective calcification arises*

TABLE 7

Loss of Ca and P in Faeces in Rickets, and its Effect on the Mineralization of Bone

	NORMAL	RICKETS
Ca lost in fæces, as percentage of intake	20–40	90–100
P lost in fæces, as percentage of intake	15	60–70
Mineral matter in bone, percentage by weight	55–60	20–30

directly from the low blood phosphate or calcium. In this sense rickets is a disorder not of the bone but of the blood In fact it has been shown that bone will calcify normally *in vitro* provided the blood-serum with which it is in contact contains adequate phosphate and calcium (Shipley 1924)

Hypervitaminosis D

This concept of the mode of action has been substantiated by observations on the effects of excessive doses of the vitamin. It has been shown that just as deficiency of vitamin D leads to a low blood phosphate

or calcium (or both) and consequent deficient calcifi-
tion so an excess of vitamin D brings about an excess
increase in the blood phosphate or calcium (or bot-
and hence over-calcification (see Harris, 1932)

TABLE 8

Blood P and Ca in Hypo- and Hyper-vitaminosis

	RICKETS	NORMAL	HYPERVITAMINOSIS
Ca, mg per 100 ml.	7	10	15
P (inorganic phosphate) mg per 100 ml	3	4	8

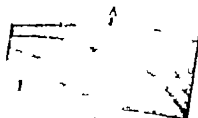








FIG. 30. Calcif[illegible]tion of bone [illegible] deficiency and excess of vitamin D

X-ray pictures of [illegible] compare density at growing end of bone
(1) Rickets inadequate calcification
(2) Normal normal calcification
(3) and (4) hypervitaminosis excessive calcification [illegible] growth cartilage

(After Harris 193[illegible])



(A)



(B)



(C)

FIG. 51 Metastatic calcification in soft tissues, in hypervitaminosis D in the rat —

(A) At root of tooth, causi g ankylosis (t C)

(B) In kidneys and

(C) I aorta (= areas stain ing black)

(*After Harris*, 1932, *and Inam*, 1929-30)

Explanation of Effect on Net absorption

There is little or no evidence that vitamin D acts directly upon the function of the alimentary canal. *It* is true that in certain circumstances administration of the vitamin can diminish the alkalinity of the intestinal contents (Zucker and Matzner 1923) This makes the calcium and phosphate more soluble and would account for the observed *increase in the net absorption But this* action does not seem general and in any event it lacks

explanation At the time of writing (1951) there is no full explanation of the chemical action of vitamin D Recent evidence based on observations with radio-active labelled elements, suggests that an initial effect of vitamin D is to cause an increased turn over of organic to inorganic phosphate in the soft tissues * The observed effect on the net-absorption may thus be secondary to this action

The Parathyroid Theory

A deficiency in the parathyroid secretion is known to cause a fall in the level of calcium in the blood and administration of parathyroid hormone results in a rise in the calcium level With excess of the hormone, calcium depositions are found in the tissues. In these respects the action of the parathyroid seems to resemble, superficially at least, the effects seen with deficiency and with excess of vitamin D respectively The theory had therefore, been propounded that vitamin D acted by stimulating the parathyroid gland This view had to be rejected however because this fundamental difference was found that whereas the parathyroid raised the calcium in the blood by withdrawing it from the bones and causing a loss to the body the vitamin D did so—as indicated above—by decreasing the loss of calcium in the fæces and thus increasing the retention in the body (see Harris 1932)

HYPERVITAMINOSIS D

Several allusions have already been made to the ill-effects produced by toxic overdoses of vitamin D The pathological changes seen in human beings are the same as those produced experimentally in animals viz. raised levels of calcium and phosphate in the blood loss of

* Cohn and Greenberg (1939)

calcium by way of the urine and abnormal calcium depositions in such sites as the kidney aorta, growth cartilage, and periodontal membrane (for further references, see Harris 1932 and later literature)

VITAMIN D AND THE TEETH

From what has been said above (p 106) it will be understood that administration of adequate vitamin D in the early years of life is important for bringing about an adequate calcification of the teeth, and hence for ensuring their normal structure the possession of sound normal teeth may therefore be expected to increase the chances of resistance to erosion

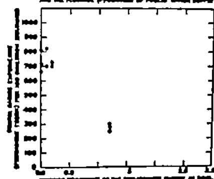


FIG 52 Incidence of dental caries in relation to fluoride content of drinking water in U.S.A.

I those areas here the fluoride content is higher the incidence of caries is less.

(*After Dean et al* 1942)

Administration of vitamin D was found by M Mellanby (1934) to *diminish* both the spread of old caries and the development of new caries in school children It did not however *entirely arrest* the disease. Thus it is clear that other factors, in addition to deficiency of vitamin D are involved in the pathogenesis of dental caries.*

It has recently been established that a sufficient intake of the "trace element fluorine, is one of the most important of these From observations on over 7 000 school children, from 21 cities in 4 different states in the U.S.A., it was shown that as the fluorine content of the public water supply increased, so the incidence of caries diminished (Dean *et al* 1942)

Too high an intake of this same trace element, fluorine, is the cause of fluorosis, an ailment which is seen in endemic form in various areas, and involves a mottling of the teeth and osteosclerosis.

This same conclusion is to be drawn also from the observation that in some regions of the globe rickets and osteomalacia are common but dental caries is almost unknown, and that in other regions caries is common but rickets is not

PHARMACOLOGICAL USES OF VITAMIN D ⊕

In Parathyroprivic Tetany In Lupus Vulgaris

The usefulness of vitamin D in preventing a precipitate fall in the level of calcium in the blood following surgical damage to the parathyroid glands, has been recognized for some time

A more recent and remarkable development is the spectacular cure of lupus vulgaris, following massive doses of vitamin D (Charpy 1943 1944 Dowling 1946 D E. Macrae 1947) For it to be effective, the amount of vitamin given has to be so high as to provoke a liability to hypercalcaemia (=hypervitaminosis) and toxic symptoms, in a proportion of cases thus careful control of the treatment is necessary

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vitamin A. If an individual in the last mentioned group (c) shows a significant improvement after being dosed with the vitamin A, but no improvement before being dosed (b)—while the controls (a) also remain unchanged —it is a reasonably sure indication that he is suffering from a deficiency of vitamin A.

In some surveys carried out in Great Britain between the two World Wars, such precautions were taken and there seemed to be an indication of some incidence of subnormality (viz *in about one third of the subjects* examined) among certain groups of inadequately fed children in poor-class areas (Maitra and Harris, 1937 Abbasy and Harris, 1938) This suggestion was confirmed by an *independent finding in some nutrition surveys* carried out at the time—in which it was shown that those children who were subnormal in the dark adaptation test had in almost all instances been those who were receiving virtually no fresh milk, either at home or at school those with better dietary histories had come in the normal group on test (Menzies 1938) Since those days social conditions have improved so much that such a degree of underfeeding must have almost vanished there has been a greatly increased consumption of milk in poor-class homes a general extension of the provision of milk in schools and of school meals not to mention the fortification with vitamin A of all margarine sold in Great Britain and a free supply of cod liver oil preparations for all expectant and nursing mothers and young children

Such evidence of deficiency *is thus no longer to be* expected in Great Britain Nevertheless it remains true to say that in the inter war period according to the independent surveys reported by Orr (1936) a considerable proportion of the population was judged to have intakes of vitamin A below the reputed optimum

In the United States of America (O'Brien 1933; Jeans and Zentmire, 1934) and in other industrial countries of the West, somewhat similar conditions appear to have applied while in parts of India (see Aykroyd and Krishnan 1936) and in other oriental regions the rate of incidence, and the severity of the deficiency have been incomparably worse.

Night blindness

Definite subjective symptoms of *night blindness* such as obtrude themselves on the patient, deserve to be clearly distinguished from the earlier and milder manifestation of *subnormal dark adaptation* which it will be understood may need special laboratory equipment for its detection. From records in the literature it is clear that this mark of the more advanced deficiency has been common in various parts of the globe, e.g. Newfoundland (Little, 1912; Aykroyd 1930) Labrador (Little, 1912) China (Pillat, 1929; Chou 1930) Guatemala (Macphail, 1929) and the Dutch East Indies (Wille, 1922). Aykroyd and others have observed that after a patient has received a large dose of vitamin A his sight may be restored to normal within so short a time as 12 to 24 hours.

It has to be borne in mind that other types of night blindness are known not related to vitamin A—for example in the hereditary defect, retinitis pigmentosa—and these do not respond to treatment with the vitamin.

Ocular Lesions

In adults a long-continued deficiency of vitamin A generally gives rise to night blindness; in children on the other hand **xerophthalmia** with or without **keratomalacia** is the more usual symptom.

The first change to be detected in xerophthalmia in man is in the **conjunctiva** which becomes dried,

vitamin A. If an individual in the last mentioned group (c) shows a significant improvement after being dosed with the vitamin A, but no improvement before being dosed (b)—while the controls, (a) also remain unchanged —it is a reasonably sure indication that he is suffering from a deficiency of vitamin A

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The first change to be detected in xerophthalmia in man is in the **conjunctiva** which becomes dried

and then wrinkled and thickened. In cases of mild deficiency of vitamin A the so-called **Bitot's spots** may also be observed at a fairly early stage. These are characteristic, chalky white specks, named after C. Bitot, the physician at the Foundling Hospital in Bordeaux who first described them in 1863

In severe cases, the xerosis of the corneal epithelium, if not treated may lead to corneal ulcers, and so to blind





FIG 57 Vitamin-A deficiency Xerophthalmia in Danish children, 1917 (*After Bloch*)

ness. If however cod liver oil or vitamin A concentrate is given in time, the cure is dramatically rapid

The classical accounts of xerophthalmia are those given by Mori in Japan (1904) and by Bloch in Denmark (1917 1924) In India, xerophthalmia is so common that it is said to have been the principal cause of blindness (Wright, 1931 Kirwan 1931) Other records refer to its prevalence in Ceylon (Nicholls, 1933 1934) in parts of China (Pillat and Chang 1932) in Yucatan (where one child in every five was a victim Carrillo 1932) in Malaya, in Newfoundland and elsewhere.

Epithelial Xerosis

The characteristic change in the skin in vitamin A deficiency described by numerous clinical observers is an excessive dryness, which may be followed later by a typical **follicular hyperkeratosis** Dry horny papules are formed which may vary in size from a pin's head to a quarter of an inch across, and which occur predominantly at first on the extensor surfaces of the thighs and forearm and may spread to other parts of the body (legs, arms, shoulders, abdomen chest, back buttocks) (Figs 58 59) However the follicular hyperkeratosis is by no means always observed in deficient subjects, and there has been some question about its specificity as as a sign of vitamin A deficiency



Fig 58 Follicular hyperkeratosis phrynoderma, toad skin in boy of 4

(After [illegible] et al 1943)

A peculiar condition of the skin seen by Loewenthal in Uganda and later by Nicholls in Ceylon has been given the name phrynoderma (Greek, from the native designation toad skin) It may be associated with lack of vitamin A, but perhaps also with other concurrent deficiencies. Loewenthal described it as consisting of a dryness of the skin with a keratinized papular eruption originating at the pilo-sebaceous follicles and occurring

on most parts of the body except the face. This condition has been shown to be very common in Ceylon (Nicholls, 1933 1934) in Uganda (Loewenthal, 1933

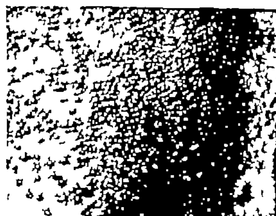




FIG. 59. Follicular hyperkeratosis, showing projecting horny spikes, before treatment (*above*) and after (*below*) This is the same case as in the previous figure.

(*After Frazer et al.*, 1943)

Mitchell, 1933 Wright, 1930) and in China (Pillat 1929 Frazier and Hu, 1931) In England, Mackay (1934) considered that, among infants minor infections

of the skin constitute a common result of hypovitaminosis-A.

Relation to Infection

See above, p 129

QUANTITATIVE REQUIREMENTS AND CURATIVE DOSAGE

Needs of Adults

A committee of the Medical Research Council (1945 1949) found that 1 300 i u of vitamin A or 2 600 i u of carotene were sufficient for a slow cure of the signs of mild deficiency in a group of human volunteers The League of Nations standard requirement of 3 000 I U for an average adult therefore seems to allow a small and reasonable margin for safety Such a supply is afforded by a daily intake for example, of 500 millilitres [7/8 of a pint] of whole fluid milk, one egg 25 grammes [9/10 ounce] of butter and a medium sized serving of a green leafy vegetable (League of Nations 1938)

Mothers Infants and Children

For pregnant and lactating women the needs are doubtless increased The requirement of infants and growing children is also greater than that of adults, weight for weight The vitamin may be supplied to infants in the form of a fish liver oil or a concentrate.

Carotene Not Quantitatively Equivalent

It must always be realized that carotene is less well utilized weight for weight than vitamin A the carotene content of a food should be divided by an arbitrary figure of about 2 to 3 in order to express approximately its equivalent biological activity in term

CLINICAL DIAGNOSIS OF DEFICIENCY DIAGNOSTIC TESTS

At least six possible indications may be sought as evidence of early vitamin A deficiency in man —

(1) diminished dark adaptation, remaining unchanged without treatment but restored to normal after administration of vitamin A (see above, p 131)
(2) xerosis in the conjunctiva (seen on inspection with a slit lamp microscope) with
(3) presence of Bitot s spots and
(4) keratinized cells in scrapings from the cornea
(5) occurrence of follicular eruptions in the skin the latter being dry and thickened
(6) low level of vitamin A in the blood

Post mortem an indication of the vitamin-A reserves of a human subject is readily obtained by chemical analysis of a specimen of the liver for its vitamin A content. This fact has proved useful for purposes of surveys, as for example, in comparisons of the vitamin A status of different sections of the community or in examining the effect of various diseases on the vitamin A economy of the body (T Moore 1937)

PROPERTIES OF VITAMIN A

Identification and Relation to Carotene

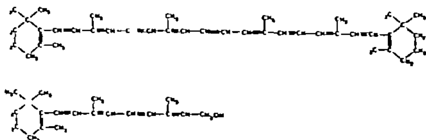
Observations by Steenbock (1919) and by Euler *et al* (1928) gave rise to the suggestion that there was some connection between vitamin A and the naturally occur ring yellow plant pigment, carotene. Then, in 1929 the experiments of T Moore led to the recognition that two distinct substances, (a) the yellow pigment carotene

and (b) the colourless vitamin A of liver oils, share vitamin A activity Carotene occurs as such in the vegetable kingdom and some or all of it is converted to

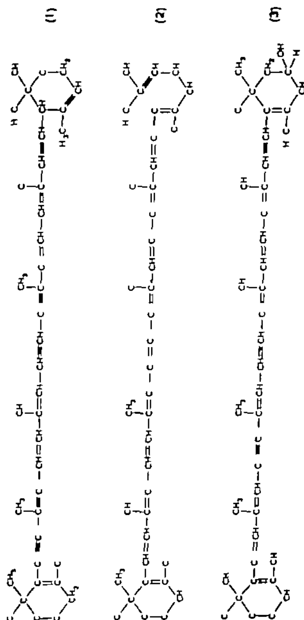


Fig. 60 Crystals of β-carotene pro-vitamin A
(*After Palmer*)

vitamin A in the animal organism The chemical relation between β-carotene and vitamin A (Karrer *et al* 1931) is shown in the formulæ below



β-Carotene above showing its relation to vitamin A (axerophthol) below

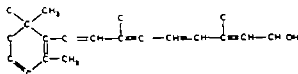


α-Carotene (1) γ-carotene (2) and cryptoxanthin (3)

Various Biologically Active Forms

It has since been shown that several other carotenoid substances in addition to β-carotene also possess vitamin A activity but the matter is not of great practical importance. **β-Carotene** has the highest biological potency of the several active carotenoids it possesses two β-ionone rings (p 145) **α Carotene** γ-**carotene** and **cryptoxanthin** which have only a single β ionone ring have about one half the activity Other active carotenoids although of little more than academic interest include **leprotene** (present in the leprosy bacillus) **echinenone** (in the sex glands of sea urchins) **myxoxanthin** (in certain algæ) and **aphanin** and **aphanicin** (in green algæ) Numerous other carotenoid pigments are inactive including for example lutein (in green leaves) and lycopene (in tomatoes)

To turn to the pre formed A vitamins (i e the alcohols) as distinct from the parent carotenoids (hydrocarbons) **vitamin A_1** is the principal form present in marine liver oils. **Vitamin A_2** occurs in some fresh water fish There are also separate **cis trans** isomers of the various active carotenes and A vitamins. In nature, the **all trans** form predominates in each case, and has the highest biological activity



Vitamin A_2.

(Tentative formula, according to R A. Morton)

due to vitamin A (alcohol or ester) plus active carotenoids (hydrocarbons) The criteria employed in the rat test may be either (a) restoration of growth, or (b) the cure of specific xerotic changes, which are best observed by means of a vaginal smear A method involving (c) measurement of the amount of vitamin A stored in the rat's liver has also been used

The distribution of vitamin A in animal tissues can also be followed histologically by means of the so-called fluorescence test. This depends on the fact that, if a tissue-slice is exposed to ultra violet irradiation those fat globules which contain appreciable amounts of the vitamin emit a distinctive fluorescence.

The chemical determination of carotene is based on a measurement of its natural yellow colour after a preliminary extraction and separation, if necessary from other carotenoids (e.g xanthophylls*) and associated pigments (such as chlorophyll) by chromatography †

In the chromatographic process di-calcium phosphate (or alumina) may conveniently be employed as the adsorbent and acetone as the extractant. Another solvent found to be useful is a mixture of light petroleum with acetone and quinol

The **international unit** adopted in 1934, is 0 0006 mg (=0 6 μg) of a standard specimen of β-carotene.

For vitamin A a corresponding unit was set up in 1949 viz 0 344 *μg of a standard preparation of* vitamin A_1 acetate, representing 0 3 μg of vitamin A_1 alcohol

Distribution of Vitamin A and of Carotene

In the animal kingdom, fish liver oils and mammalian livers provide the best sources of vitamin A Fish body oils and beef and mutton fats are but poor carriers. Ordinary margarine, as formerly supplied to the public,

Carotenols." †For Bibliography see Society of Public Analysts, 1950.

was inactive, but during the Second World War th fortification of margarine with vitamin A was mad compulsory in Great Britain and this no doubt helpe to prevent any deficiency among the population

In the plant kingdom pre-formed vitamin A does n occur as such Most leafy vegetables are however ric in β-carotene, and hence highly active. The activit often runs parallel with the degree of greenness. Amon root vegetables the carrot and sweet potato are especiall active most others are poor Certain yellow fruits contai significant quantities. Red palm oil has a very hig carotene content most other vegetable oils contain littl or none.

Dairy products are distinctive in owing their vitamin activity partly to the vitamin itself and partly to caroten Milk and butter are important as sources of vitamin in the national diet egg yolk is less important becau of the relatively smaller amounts consumed

Origin of Vitamin A in Nature

The ultimate source of all the vitamin A in the anim kingdom whether in mammals or in fish can be trace back to the carotene synthesized by plant life terrestri or aquatic. Thus, the vitamin A in a helping of ox live consumed by a human being derives from the caroten of the pasture. Similarly the vitamin A in our dose fish liver oil originates in the carotene (or other activ carotenoid pigment) elaborated by the minute plant li of the sea this is devoured by lowly Crustacea, mollus or small fish which in turn form the prey of larger fis such as the cod or halibut We thus have a vitamin chain

Whale liver is extremely rich in vitamin A, and the has been some question whether the whale gets its vitam preformed or as provitamin-carotenoids. Kon an

due to vitamin A (alcohol or ester) plus active carotenoids (hydrocarbons) The criteria employed in the rat test may be either (a) restoration of growth or (b) the cure of specific xerotic changes, which are best observed by means of a vaginal smear A method involving (c) measurement of the amount of vitamin A stored in the rat s liver has also been used

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*Carotenols. †For Bibliography see Society of Public Analysts, 1950.

FIG. 62. Interrupted pregnancy in vitamin-E deficient rat.

The curve shows that the pregnant rat, after the first mating, failed to gain weight normally in consequence of the resorption of the foetuses (marked by crosses). No live young were born. In contrast, after a dose of vitamin E, and a second mating, pregnancy proceeded normally, the weight curve rising with growth of the foetuses until the precipitate fall at the time of birth.

(*Reproduced after Harris*, 1936)

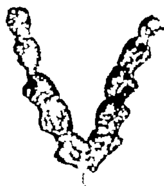




FIG. 63. Pregnant uterus of vitamin E deficient rat (*right*) showing arrested development of foetuses, as compared with control rat (*left*).

(*After Wright*)

(3) a pigmentation and discoloration in the uterus and elsewhere apparently associated with a degeneration of muscle fibres
(4) a degeneration of the epithelium of the convoluted tubules of the kidney
(5) a so-called exudative diathesis and
(6) dental depigmentation



FIG 64 Paralysis in [illegible] amin-E deficiency in rat.
The rat on the right had received [illegible] itamin E free diet, that on the left the same diet supplemented with [illegible] vitamin E concentrate.
(*After Mart[illegible] and Moore 193[illegible]*)

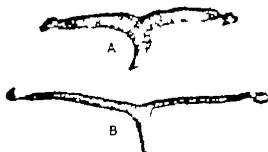


FIG 65 Uterus of rat in [illegible] itamin-E diet series showing abnormal pigmentation (above) as compared with [illegible] normal rat receiving [illegible] itamin E below
(*After Martin and Moore 193[illegible]*)

Weight of rat in gm.

210
200
190
180
170
160

Mated 1
(1) Resorption gestation
Mated 2
(2) Normal pregnancy
Litter of 8 young born
No vitamin E given
Dose of vitamin E given

0 10 20 30 40 50 60 70
Days

FIG 62 Interrupted pregnancy in vitamin-E deficient rat.

The curve shows that the pregnant rat, after the first mating, failed to gain weight normally in consequence of the resorption of the foetuses (marked by crosses) No live young were born. In contrast, after dose of vitamin E, and second mating, pregnancy proceeded normally the weight curve rising with growth of the foetuses until the precipitate fall at the time of birth.

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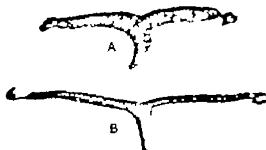


FIG 65 Uterus of rat in vitamin-E deficiency showing abnormal pigmentation (above) as compared with normal rat receiving vitamin E (below)
(After Martin and Moore 1939)





FIG 66. Degeneration of the convoluted tubules of the kidney in vitamin-E-deficiency in rat.

(After Martin and Moore)

Microphotographs, showing, above, degeneration of convoluted tubules, and below for comparison, normal structure in control rat receiving vitamin E.

In Various Species

Avitaminosis E has been examined mostly in rats but there are some records of symptoms of deficiency seen also in several other species, including chicks (particularly used in the study of the *exudative diathesis* mentioned above, and of a characteristic *encephalomalacia*) and cattle A section of veterinary opinion has supported the contention that habitual abortion in cows may sometimes respond to vitamin E (Vogt Möller and Bay 1931)

In Clinical Medicine

Claims have been made for vitamin E in the treatment of habitual abortion in women (e.g Vogt Möller 1933 1936 Tanberg 1936 Currie, 1936 1937 Young 1937) but there still seems to be the lack of a directly controlled test in which equal numbers of women treated with vitamin E have been compared with an equal number of controls left undosed However statistical comparison of existing records is said to speak conclusively in favour o the value of vitamin E in such cases (Bacharach, 1940)

The use of vitamin E in treatment of muscul dystrophy was suggested by the results of work on anima nevertheless great caution is needed in the interpretati of clinical claims, and earlier hopes have not b sustained Similar remarks apply to the alleged ind tions for vitamin E therapy in cardiac disease an treatment of gastric ulcer

Methods of Assay

Vitamin E is determined in foodstuffs either b logical or chemical methods In the former the procedure is to ascertain the average dose w needed to secure fertility in 50 per cent of group of female rats which would otherwise a

resorption gestations as the result of deficiency of vitamin E.

Chemical tests depend on the reduction of (a) ferric salts to ferrous, or the comparable reduction of (b) auric salts colorimetric or electrometric methods are used *

International Unit and Standard

The international unit (1 i.u.) is 1 mg of the International Standard Preparation, which consists of a specimen of purified, synthetic racemic α tocopheryl acetate.

Distribution in Foodstuffs

The best sources of vitamin E include wheat germ and rice germ oils cotton seed oil and green leaves (e.g lettuce) Cod liver oil and other fish liver oils although so richly endowed with the two other important fat soluble factors (vitamin A and vitamin D) are generally almost devoid of E Indeed substances present in cod liver oil may exert a destructive action on vitamin E in foods or in the animal organism

Isolation of α tocopherol

In 1936 Evans and his co-workers isolated the vitamin in a crystalline form and gave it the name α tocopherol It has the formula $C_{29}H_{50}O_2$, and may be regarded as built from trimethyl-quinol and phytol molecules. The presence of the latter grouping in vitamin K_1 (p 163) may be noted

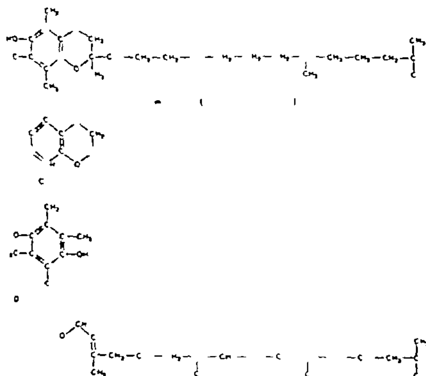
β γ- and δ Tocopherols

β- γ- and δ-Tocopherols have structures closely related to α tocopherol, differing only in the omission of

* Emmerie and Engel, 1938 and Karrer and Keller 1938, respectively For review of chemical methods, see Eden and Booth, 950

either one or of two of the three methyl groups α-Tocopherol is 5,7,8-trimethyl tocol β-tocopherol 5,8-dimethyl tocol and γ-tocopherol 7,8-dimethyl tocol δ-Tocopherol is 8-monomethyl tocol

The structural relations are set out below

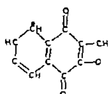


α-Tocopherol (5,7,8-trimethyl tocol) showing structural relationship to chroman, duroquinol and phytol.

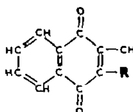
Certain other related substances have also been synthesized and show some degree of biological activity

had been found necessary because of their poor absorption either to inject them or to administer bile salt simultaneously The introduction of this new alternative, vitamin K_3, thus represented a considerable advance. From vitamin K_3, also can be prepared various water soluble derivatives which are useful when treatment by injection is desired

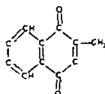
2 Methyl 1 4 naphthoquinone is now official in the British and U S Pharmacopoeias, where it has been given the names **menaphthone** and **menadione** respectively



Phthiocol.
(2-Methyl-3-hydroxy-1,4 naphthoquinone.)



General formula for K vitamins.



Menaphthone" ("Menadione")
(2-Methyl-1,4-naphthoquinone.)

Mode of Action and Properties of Vitamin K

Little is known about the mode of action of vitamin K. Prothrombin itself has no vitamin K action nor does the vitamin possess prothrombin activity A suggestion that, biochemically vitamin K exerts its influence by its ease

of oxidation into phthalic acid has not been confirmed All that can be said is that in the absence of vitamin K, the liver is unable to carry on its normal task of elaborating prothrombin.*

Anti Vitamin K

A naturally occurring sweet clover disease in cattle has been traced to the action of dicoumarol a substance which is chemically related to vitamin K, and which 'blocks the action of the latter so causing hypoprothrombinæmia, and consequent hæmorrhage. The disorder is cured by vitamin K

Dicoumarol has been employed in medicine for the treatment of thrombosis. It has its obvious risks—the possibility of inducing excessive hæmorrhage—and so must be used with great caution

Clinical Uses

Vitamin K itself finds clinical application in the prevention and cure of those hæmorrhagic conditions which are due specifically to a low prothrombin value. These fall into three categories, to be discussed more fully below namely (1) a hæmorrhagic state in new born infants, (2) hæmorrhage occurring after surgical treatment of obstructive jaundice and (3) hæmorrhage associated with steatorrhœa or with gastro-intestinal obstruction, which cause a faulty absorption of the vitamin.

Other hæmorrhagic states not associated with low prothrombin e.g hæmophilia are of course not susceptible to treatment by vitamin K. Again a low prothrombin figure may sometimes be due to liver damage rather than to any lack of vitamin K and in such cases administration of the vitamin may be without effect

* Cf. Addendum, p. 223.

Vitamin K and Neo Natal Hæmorrhage

In the new born infant, a transient hypoprothrombi-æmia frequently occurs on the second to third day afte birth and may persist for five to six days. This is th cause of a fairly common condition, a hæmorrhagi state seen from two to six days after birth, in which th hæmorrhages may be observed in the gastro-intestina tract (discoloured stools being passed) from the cord



FIG. 67 Hæmorrhage in new-born child due to vitamin-K deficiency (hypoprothrombinæmia)

(After Grossman)

Hæmorrhages can be seen in the skin of the upper arm, shoulder and umbilical cord in this three-day-old infant. Treatment with vitamin K effected cure.

in the nose and palate, and in the genito-urinary tract. It is now thought that intracranial hæmorrhages also may sometimes be due to vitamin K deficiency

It would be an obvious fallacy to regard all hæmorrhages in the new born as necessarily due to hypoprothrombinæmia, and therefore amenable to treatment with vitamin K. Nevertheless statistics tell a striking

:cess in the prevention of neo-natal hæmorrhages
dication with vitamin K

:planation of the transient hypoprothrombin
l the consequent hæmorrhage is thought to be
normal intestinal micro-flora, which ordinarily
s vitamin K by a symbiotic process does not
iance to become firmly established in the new
nt until after the ingestion of food Prior to
e limited transfer of the vitamin no doubt occurs
mother to the fœtus via the placenta

K Therapy for the New born

ly may consist either of (1) pre natal treatment
ther or (2) the prophylaxis or curative treatment
v born infant itself The former is to be preferred
een shown that administration of vitamin K
er day of 2 methyl 1 4 naphthoquinone) to the
or one month prior to the calculated time of
is completely effective in preventing hypopro-
inæmia Failing such pre natal treatment, vitamin
be given to the mother at the onset of labour
vely the new born infant may be given vitamin
iylactically (1 4 mg of 2 methyl 1,4 naphtho-
per day either orally or by injection) Of 400
ints treated with vitamin K only 1 per cent

TABLE 10

hylaxis of Neonatal Hæmorrhage by Vitamin K

(From data of Waddell and Lawson, 94)

Infants ...ed	Treatment	Incidence of Hæmorrhage, per cent
100	Vitamin K (administered t[illegible] child t birth)	1
9	Nil	10

developed hæmorrhages, whereas of 219 not treated 10 per cent developed hæmorrhages (Waddell and Lawson 1940) If hæmorrhages should have occurred in the absence of prophylactic treatment, a suitable dose for curative treatment is 1–5 mg of 2 methyl 1,4 naphthoquinone to be given to the infant every 12 hours.

In Obstructive Jaundice

The second important use of vitamin K is to raise the prothrombin level and hence prevent hæmorrhage after the surgical treatment of obstructive jaundice. The explanation of this use of vitamin K is as follows The biliary obstruction—which is the cause of the jaundice—results in an absence of bile from the intestinal tract, and hence the vitamin K is not absorbed In consequence, there is a low prothrombin figure, and so hæmorrhages are liable to result. Formerly this hæmorrhage was the cause of a high mortality rate in the surgical treatment of obstructive jaundice, but since the introduction of vitamin K the outlook has been transformed this attendant hazard having been almost abolished Treatment with the vitamin is now given as a pre-operative routine. Preparations of natural vitamins K_1 or K_2 were used at first being administered with bile salts to ensure their assimilation (cf. above, p 164) Now however the synthetic product, 2 methyl 1,4 naphthoquinone, is used *instead It may itself be given* by mouth, or injected in the form of certain water-soluble derivatives (p 164)

Conditioned Deficiency Failure in Absorption or *Obstruction*

Hæmorrhage, due to low prothrombin values may also be caused by a failure to absorb the vitamin K, either because of an obstruction or for some other reason Vitamin K deficiency *of this type* has been noted

particularly in association with (a) steatorrhoea, including that occurring in (b) sprue, and (c) sometimes also in patients with intestinal obstruction Treatment with vitamin K is indicated in such cases, a usual dose again being from 1–4 mg of 2 methyl 1 4 naphthoquinone

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CHAPTER IX

THREE ADDITIONAL B_2 VITAMINS

RIBOFLAVIN AND HUMAN CHEILOSIS PYRIDOXIN AND RAT PELLAGRA PANTOTHENIC ACID AND CHICKEN PELLAGRA

It is convenient to discuss together in this Chapter these three vitamins which are often found associated together in foodstuffs and which in their **earlier history** were, one after another each in turn mistaken for the true pellagra preventing factor (see pp 16-17 47)

They are —

(1) **riboflavin** first demonstrated to be a vitamin by its growth-effect for rats a deficiency is now recognized to be the cause, in humans of a condition known as cheilosis (pp 171–2)

(2) **pyridoxin** or vitamin B_6, lack of which was shown to be responsible in rats for a severe skin disease, first miscalled rat pellagra

(3) **pantothenic acid** found to prevent a disease in poultry formerly known as chicken pellagra

Later investigations have proved that these vitamins are of importance for various other species also—e.g both riboflavin and pantothenic acid are needed by dogs by rats and by pigs as well as by poultry

(1) RIBOFLAVIN

Discovery

Riboflavin a water-soluble, fluorescent yellow-coloured substance was detected first in milk, by Winter Blyth in

1879 and subsequently shown to occur also in egg liver kidney and other tissues. Its role as a growth promoting vitamin for rats was established by Kuhn György and Wagner Jauregg in 1933



FIG 68 Crystals of riboflavin.
(After Kuhn)

Nomenclature

Riboflavin was formerly called lactoflavin (or ovoflavin or hepatoflavin etc according to its origin) and is still sometimes known as vitamin B_2. (Strictly speaking this last term was correctly used only when riboflavin was still the sole known B_2 vitamin—that is before the latter was found to be a complex see pp 16 47)

Clinical Significance

A deficiency of riboflavin in man is now known to be associated with cheilosis (lesions on the lips) together with an angular stomatitis (with cracking scaliness and maceration at the corners of the mouth, leading to fissuring) and a specific type of glossitis (the magenta tongue) A seborrhoeic dermatitis and corneal changes —opacity and vascularization—have also been described



FIG. 69. Lesions of lips, tongue and eyelids in riboflavin deficiency in man.

There are deep fissures at the corners of the mouth (angular stomatitis); the lips are cracked and inflamed (cheilosis), the tongue is cracked and of fiery-red colour; and there is dermatitis of the eyelids which are macerated and stuck together (blepharospasm).

(After Hou; and Sydenstricker et al.

Deficiency in Various Species

In poultry nutrition, riboflavin is of practical importance as preventive of a deficiency disease known as curled toe

In dogs a riboflavin-deficiency disease, 'yellow liver has also been described Rats deprived of riboflavin suffer from an eczematous condition of the skin, and corneal lesions may develop Pigs also have been shown to need riboflavin For many species of micro-organisms riboflavin is a growth promoting factor

Human Requirement

The daily requirement (preventive) is probably about 1–2 mg

Estimation in Foodstuffs Assessment of Nutritional Status

The fluorescence of riboflavin* is widely used as a method for its quantitative estimation in foods and tissues. Other methods depend (a) on growth of rats, or (b) on microbiological tests, in which lactic acid production is measured (Snell and Strong, 1939, and subsequent literature) The nutritional status in man can be assessed by the urinary excretion after a loading test (cf p 75)

Distribution in Foods

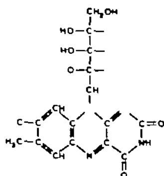
Foods rich in riboflavin include yeast, liver kidney milk and eggs (white and yolk) Beer also contains significant amounts

Biochemical Role

Riboflavin is known to have one important physiological function as a component of certain co-enzymes, namely

* Euler and Adler 933 1934 Kuhn, Gyorgy and Wagner Jauregg 1933 and an extensive later literature.

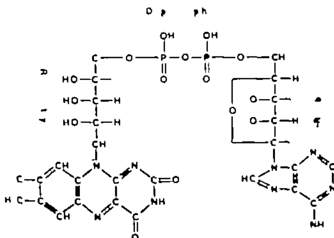
the *iso*alloxazine adenine dinucleotides. The latter in combination with various specific proteins form **flavoproteins** which act as catalysts by transporting hydrogen in a variety of oxidative enzyme reactions



Riboflavin.
(Dimethyl-9-D-ribityl-*iso*alloxazine.)



*Iso*alloxazine.



Riboflavine co-enzyme
(*Iso*alloxazine adenine dinucleotide.)

Deficiency in Various Species

In poultry nutrition, riboflavin is of practical importance as preventive of a deficiency disease known as curled toe

In dogs, a riboflavin-deficiency disease, yellow liver' has also been described Rats deprived of riboflavin suffer from an eczematous condition of the skin, and corneal lesions may develop Pigs also have been shown *to need riboflavin For many species of micro-organisms* riboflavin is a growth promoting factor

Human Requirement

The daily requirement (preventive) is probably about 1–2 mg

Estimation in Foodstuffs Assessment of Nutritional Status

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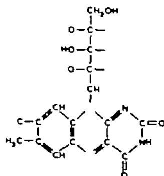
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Biochemical Role

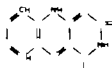
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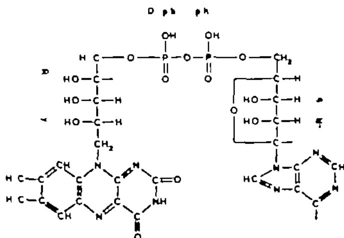
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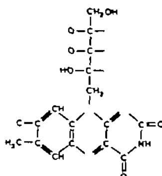
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Biochemical Role

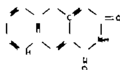
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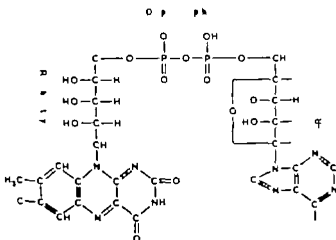
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Riboflavin.
(Dimethyl-9-D-ribityl-isoalloxazine.)



Isoalloxazine



Riboflavine co-enzyme
(*Isoalloxazine*-adenine dinucleotide)

Form of Occurrence

In milk a greater part of the riboflavin is present in the free state, whereas in other materials such as yeast, liver and plant tissues, it seems to occur mostly in the combined form

(2) PYRIDOXIN (VITAMIN B_6 ADERMIN)

Deficiency in Various Species

Deficiency of pyridoxin in rats as described by György (1934) is marked by a florid symmetrical dermatitis of the

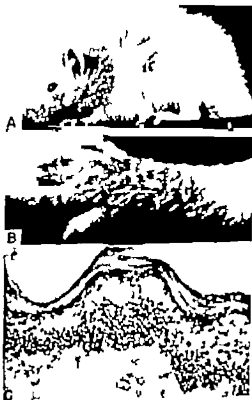


Fig 72 Pyridoxin deficiency in rat.

(After Sullivan and Nicholls)

The fur is normal (A) but there is dermatitis of the extremities (B); and, microscopically (C), hyperkeratosis can be seen, with acanthosis, and epithelial proliferation just above the basal cell layer

extremities. This may be accompanied by hæmaturia and sometimes by epileptiform seizures The last mentioned sign is seen also in pigs, together with a microcytic anæmia The deficiency has been studied also in chicks

Man appears to need pyridoxin and its deficiency is perhaps associated with a cheilosis similar to that seen with deficiency of riboflavin

Structure

Pyridoxin resembles nicotinamide in being a pyridine derivative. It was isolated and its structure determined

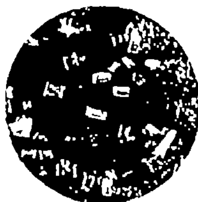
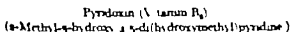


FIG. 73. Crystals of pyridoxin (vitamin B_6)
(*After Bucknell and Prescott*)

in 1939 and it was soon afterwards synthesized (S A. Harris and Folkers 1939 Kuhn *et al* 1939) Its structure is shown below



Pyridoxin (Vitamin B_6)
(2-Methyl-3-hydroxy-4 5-di(hydroxymethyl)pyridine)

Distribution and Estimation

Vitamin B_6 is found in many different types of food stuffs Among the best sources are yeast, liver pulses and cereals A noteworthy fact is that it occurs in appreciable amount in maize and maize products, which, although productive of pellagra, will therefore cure vitamin B_6 deficiency in animals

Tests on rats have been most commonly used for its estimation. An alternative assay is with chicks. Chemical and microbiological methods have also been described (e.g by Scudi 1941 and by Snell, 1950 respectively)

Biochemical Function

The physiological role of pyridoxin appears to be in relation to protein metabolism Several derivatives have biological activity The corresponding aldehyde, **pyridoxal** is concerned as a co-enzyme (co-decarboxylase) in the decarboxylation of certain amino acids (Gale, 1946) and like the amine, **pyridoxamine** in biological transaminations (in the so-called aminophorase system) These substances, related to pyridoxin function in the enzyme systems in a phosphorylated form. A third derivative **pyridoxic acid** has been shown to occur in human urine

Suggested Medicinal Use

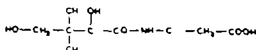
It has been claimed that pyridoxin is of use clinically in combating agranulocytosis after therapy *with thiou*racil or sulphonamide (Cantor and Scott, 1945 Fishberg and Vorzimer 1945 Piney 1946) However similar claims have been made for various other supposed remedies and as such cases tend to improve anyway irrespective of treatment, the evidence is difficult to assess.

(3) PANTOTHENIC ACID

This vitamin is concerned in preventing a disease in poultry (Ringrose Norris and Heuser 1931) formerly called chicken pellagra on the mistaken assumption that it was analogous with human pellagra The vitamin was afterwards shown to be identical with a previously described nutrient for **micro organisms** called pantothenic acid (R. J Williams, 1939) Its identity was then established with a **'filtrate factor** for rats (By filtrate factor is meant a component of the vitamin B_2 complex differing from nicotinamide riboflavin and pyridoxin by not being adsorbed on fuller's earth but on the contrary passing through into the filtrate from this adsorption)

Chemical Constitution

Pantothenic acid has the following structure (Williams and Major 1940) —



Pantothenic acid (γ-dihydroxy-β,β-dimethyl-butyryl-β -alanide

Deficiency in Various Species

Deficiency of pantothenic acid in poultry gives rise to a characteristic dermatitis In rats there is growth failure associated with dermatitis, nose bleeding and the occurrence of a sticky exudate in the eyelids, depilation around the nose, achromotrichia (a greying of the black hairs) and adrenal lesions Dogs and pigs and almost certainly human beings also require pantothenic acid





FIG 74. Greying of fur (achromotrichia) in pantothenic-acid deficiency in a rat and (to right) microscopic section of the skin to show the characteristic dilatation of the hair follicles.

(After Sullivan and Nicholls)

Clinical Uses

It has lately been claimed that a deficiency disease common in parts of Southern India, the so-called burning feet syndrome, is cured by pantothenate (Gopalan 1946) Also glossitis associated with Addisonian pernicious anæmia, or with steatorrhoea, has been found to respond on different occasions to treatment with single synthetic vitamins of the B complex, including nicotinic acid or folic acid but more especially to pantothenate (Brown 1949)

Mode of Action

Pantothenic acid appears to be the component of a co-enzyme system concerned in various acetylation reactions (Lipmann *et al* 1947)

Assay and Distribution in Foods

The vitamin occurs in yeast and liver and various other foods It is determined by means of microbiological tests * or with chicks or rats.

* See Snell, 1950.

OTHER B VITAMINS

Other vitamins of the "B" group including

p-aminobenzoic acid
inositol
choline
biotin (vitamin H)
folic acid

are discussed in the next Chapter

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CHAPTER X

OTHER VITAMINS

In this chapter the following additional vitamins w[illegible] be described

Known by letter or by name

Vitamin F better referred to as the **nutritionall[y] essential unsaturated fatty acids**

Vitamin H now generally known as **biotin**

Vitamin P *(so-called 'permeability' factor)*

Known by name

Choline

Inositol

***p*-Aminobenzoic acid**

Folic acid

Strepogenin

The order in which they are here taken is approximately that of their discovery Folic acid is the only one of these vitamins which has so far proved of much clinical interest

New Vitamins

Other names which have appeared almost for the first time as this book was still in preparation include —

Animal protein factor (or factors) Zoopherin

Vitamin B_{12}

ESSENTIAL FATTY ACIDS (VITAMIN F)

Experimental Deficiency in Rats

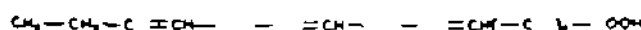
Rats fed on synthetic diets artificially freed from all traces of fat develop a disease (Burr and Burr 1929 1930) which is characterized by (1) a scaliness of the tail sometimes leading to caudal necrosis (2) irregularities in reproduction and (3) kidney lesions The disease is due to deficiency of the nutritionally essential unsaturated fatty acids linoleic acid linolenic acid and arachidonic acid

Clinical Enquiries

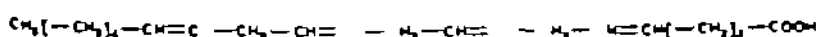
Attempts to produce the deficiency state in human volunteers have failed and it would indeed be hard to produce a diet devoid of these components Nevertheless vitamin F has been claimed to be a skin vitamin and has been exploited for use in cosmetics! A more serious case has been made for its having had a possible beneficial result in some eczematous conditions but a final judgment can scarcely yet be given

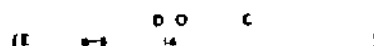












I tamins
(N utritionally essential unsaturated fatty acids)

BIOTIN (VITAMIN H)

Synonyms

Biotin was the name first given to a growth factor needed by yeast* and other micro-organisms. Later it was found to be identical† with the so-called **vitamin H**‡ (=Haut [skin] Faktor, or anti egg white injury factor § for rats and other animals) and with **coenzyme R** *(growth factor for the micro-organisms in the nodules of legumes)* ||

Properties

Biotin protects rats and other experimental animals (e.g chicks) from seborrhoeic skin lesions and other abnormalities when they are fed on a special diet containing raw egg white as an ingredient. The latter contains *a protein like substance or anti vitamin* **avidin** (Eakin Snell and Williams 1940) which inactivates the vitamin but is destroyed by cooking

Clinical relations are still obscure, although biotin deficiency has been produced experimentally in the human subject by means of a diet containing large amounts of raw egg white (Sydenstricker *et al* 1942) and there is said to be at least one record of a primary deficiency having been seen resulting from the use of a similar diet (R H Williams, 1943)

Chemical Constitution and Assay

The molecule of biotin can be regarded as derived from molecules of urea, thiophen and valeric acid

* Kögl (1935)
† György *et al* (1940)
‡ György (1931 1939)
§ Bateman (1916) Boas (1927)
|| Allison *et al* (1933)





Fig. 73. Biotin defic[illegible] in r[illegible]

([illegible])

The characteristic features [illegible] the [illegible] skin is covered with [illegible] scales, the loss of hair and the peculiar humped po[illegible] and abnormal [illegible]



FIG 76 Histology of skin, in biotin deficiency in rat.

(After Sullivan and Nicholls)

The microscopic section shows hyperkeratosis, and dilatation of the orifices of the hair follicles, resulting in peculiar finger-like appearance of the epithelium. The underlying corium is little changed

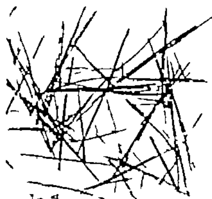
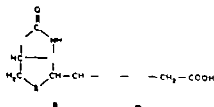
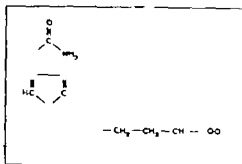


FIG 77 Crystals of biotin.

(After du Vigneaud et al)

(du Vigneaud *et al* 1942) Microbiological methods are used for its estimation Biotin occurs in yeast liver and various other materials





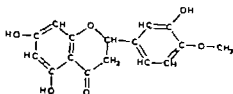
Biotin (Vitamin H) showing [illegible] structural deri[illegible] from [illegible] ur[illegible] thiophen and [illegible] aleric acid

Biochemical Role

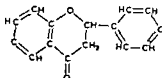
Tests with micro-organisms have suggested that biotin may be concerned, perhaps indirectly, in ammonia assimilation (as in the conversion of oxalacetic acid to aspartic acid) and perhaps more directly as a co-enzyme in the fixation of carbon dioxide (i.e. carboxylation in the oxalacetate system).

VITAMIN P AND CAPILLARY FRAGILITY

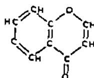
Vitamin P or 'citrin' (a group of substances including hesperidin and some related glycosides) was first regarded as a factor concerned, together with vitamin C, in preventing scurvy in guinea pigs or in sparing the needs



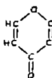
H



F



C



P

Hesperitin, and its parent substances, flavanone, chromone and γ pyrone (Hesperitin, in the form of its glycoside hesperidin, is regarded as one form of vitamin P)

for vitamin C * This, however has never been satisfactorily confirmed Later vitamin P was claimed as a factor controlling the fragility of the capillary blood vessels in man This again has been disputed. If it

* Armentano, Bentsath, Benes, Rusznyak and Szent-Györgyi (1936); Rusznyak and Szent-Györgyi (1936) Bentsath, Rusznyak and Szent Györgyi (1936) Bruckner and Szent-Györgyi (1936, 1937) Szent-Györgyi (1938)

is maintained that some cases of scurvy apparently needed both vitamins C and P for healing on the other hand it can also be confidently asserted that many cases have been cured by exhibition of vitamin C alone without additional 'vitamin P'! Thus the issue is still a controversial one.

To avoid confusion an American Committee in 19[illegible] has recommended that the term vitamin P should no longer be employed

CHOLINE A TRANSMETHYLATING AGENT

Deficiency in Animals

Deficiency of choline in rats (Best *et al* 1932) and other experimental animals (dogs and rabbits) results in a fault

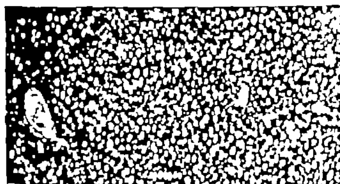


FIG. 78. Fatty infiltration of liver in choline deficiency rat
(*After Follis*, 1948)

Almost every cell is seen to be distended with fat globules Magnification [illegible]

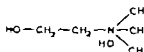
in fat transport and consequently in fatty liver (sometimes accompanied by cirrhosis) as well as by hæmorrhages in the kidney paralysis, and other ill effects Fatty livers produced in dogs by removal of the pancreas, can also

be prevented by administration of choline. In chicks deficiency of choline may cause a condition known as "slipped tendon" or "perosis" (a similar effect being produced also by lack of biotin or of the "trace element" manganese).

Physiological Significance

Biochemically choline functions as a transmethylating agent, that is, it provides a source of labile methyl groups.* As such it is replaceable by the amino-acid methionine, or by betaine. It is thought that the increased requirement for choline brought about by depancreatization (see above) is due to a shortage of methionine which would be normally liberated from the proteins of the diet by the action of pancreatic juice.

Possible clinical applications still remain to be defined, although its use has been proposed to control a negative nitrogen balance in conditions similar to those in which methionine or a high protein diet have been found effective—as during recovery from injuries and burns and after fever.



Choline
(Trimethyl-hydroxyethyl-ammonium hydroxide.)

INOSITOL

A Nutrient for Micro-organisms and (?) for Mice and Rats

Inositol is identical with a growth factor formerly called Bios I (see Table 11, p. 192) needed for the growth

* du Vigneaud *et al.* 1939.

of yeast* and other micro-organisms In mice† a deficiency is said to cause baldness in rats‡ a similar condition spectacled eye (denudation of hair) has been described



FIG. 79. Baldness caused by inositol deficiency mouse
(From Fell ...)

The hair has been lost from the body, and remains only on the head and extremities

Deficiency in man is unknown As to its biochemical action, inositol may possess lipotropic properties It is possible, however that inositol is merely a secondary nutrient i.e. it may stimulate the micro-flora of the intestine to produce other nutrients needed by the host

Recently it has been found that inositol is a component of the enzyme, **α amylase** present in liver (Lane and Williams, 1948) The nutritional significance of this finding is still awaited

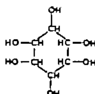
Another point of interest about inositol is that as its hexa phosphate derivative **phytic acid** it functions as a rachitogenic agent antagonizing vitamin D pp 100–1)

* Eastcott (1928)

† Woolley (1940a, b)

‡ Pavcek and Baum (1941) Nielsen and Black (1944)

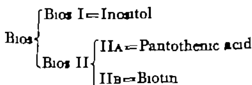
The insecticide **Gammexane** (the *gamma* isomer of benzene hexachloride) is structurally related to inositol. The suggestion has been made that it may function by blocking inositol, that is, by acting as an anti vitamin, but this is questioned



Inositol.
(Cyclohexanehexol benzen hexahydroxide.)

TABLE 11

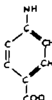
Scheme of Nomenclature of Bios Fractions


Bios { Bios I = Inositol
Bios II { IIA = Pantothenic acid
IIB = Biotin

p-AMINOBENZOIC ACID

A Growth Factor for Micro organisms

p-Aminobenzoic acid (or 'PABA') is a growth factor for various micro-organisms



p Aminobenzoic acid.

Relation to Sulphonamides and Infection

Of great interest is the fact that drugs of the sulphonamide class exert their bacteriostatic action by blocking the bacterium's supply of PABA Indeed it was a study of the mode of action of the sulphonamides which first led to the recognition of PABA as a nutrient for micro-organisms (Woods, 1940)

In the opposite direction PABA can negative the bacteriostatic action of the sulpha-drugs. Claims have been made that large doses of PABA itself may exert an anti infective action in animals in some circumstances (see Dutcher and Guerrant, *Annual Review of Biochemistry* 1946) but doubts have been expressed about this

Deficiency in Mammals

PABA has been said to prevent achromotrichia (cf p 180) in some species (Ansbacher 1941) but experi

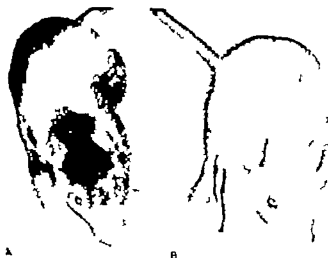


Fig. 60. Greying of the hair (achromotrichia) in *p*-aminobenzoic acid deficiency in rat.

(*From* Fells *after* S. Ansbacher)

A. After four [illegible] on deficient diet
B. Control rat, cured by *p* aminobenzoic acid

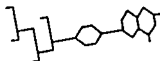
pteroyl triglutamic acid,* and **pteroyl heptaglutamic acid** (= B conjugate) *A fourth variant is the* parent substance, **pteroic acid** i e. the combined pterin and *p*-aminobenzoic acid molecules without any glutamic acid † Other pterin derivatives may also be active.

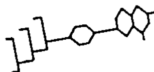
Pteroic acid, pteroyl-glutamic acid, and pteroyl-diglutamic acid ("diopterin")

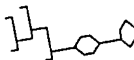
Also known as "teropterin, or as the "fermentation *L. casei* factor — not to be confused with the *L. casei* factor proper which is pteroyl-glutamic acid

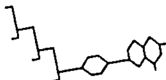
† For literature, see Annual Review of Biochemistry 1947 1948.

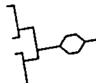
PTERIDINE DERIVATIVES



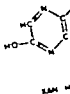


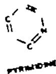






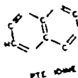
Skeleton formulæ of five possible isomers of pteroyl-triglutam...











The pteridine ring system (formed by fusion of pyraz... showing also structural relation to xanthopterin ...

Action in Macrocytic Anæmias

Great interest was aroused in the clinical possibilities of folic acid when it was found to produce a hæmatopoietic response in Addisonian pernicious anæmia, and in some other macrocytic anæmias in man (Spies 1946) A note of warning was soon sounded however when it was reported that, unlike liver extracts, folic acid was without effect in checking the cord degeneration in pernicious anæmia and hence if used experimentally in treatment it was essential that a watch should be kept for posterolateral cord signs (Spies and Stone, 1947) Other observers emphasized indeed that the use of folic acid in place of liver in the treatment of pernicious anæmia, was to be condemned for while giving as good a hæmatological response, it might actually hasten the signs of involvement of the central nervous system (Davidson and Girdwood 1947 Heinle and Welch, 1947 Wilkinson *et al* 1946 Ross *et al* 1948) (See further below under vitamin B_{12}, pp 203 205)

Folic acid has, however been used with remarkable success in the treatment of the macrocytic anæmia associated with tropical sprue, and of some other nutritional macrocytic (megaloblastic) anæmias (cf p 204)

Possible Relation to Tumour Formation

Administration of pteroyl triglutamic acid was claimed by Leuchtenberger and his co-workers in 1945 (see further Lewisohn *et al* 1946) to have caused a regression of spontaneous breast tumours in mice, folic acid itself (pteroyl glutamic acid) being without effect. These findings however could not be substantiated by some later investigators. On the other hand it has been found that the development of Rous sarcoma in baby chicks can be inhibited by a **deficiency of folic acid,** or better by the administration of certain **structural analogues**

of folic acid—which act by antagonizing it (Woll 1948 Little *et al* 1948)

In clinical trials in man one such analogue especially **aminopterin** has been used for the treatment of acute leukæmia and has resulted it is claimed in clinical improvement and in inducing short remissions at a rate far in excess of that at which they could be expected to occur spontaneously (Farber *et al* 1948 Meyer 1948 Blood 1948) There may perhaps be some rational basis for such a treatment of leukæmia with folic acid inhibitors since folic acid itself appears to be needed for the development of the white cells and its administration is said sometimes to accelerate the leukæmic process.

Preliminary reports have also appeared of the use of teropterin in treating human neoplastic disease the cautious claim is made that it relieved pain and caused a sense of well being (Lehv *et al.*, 1948)

It is necessary to emphasize however that these trials of folic acid and of folic acid antagonists in neoplastic disease are still (1948-51) in the early experimental stage and final judgments cannot yet be passed *

It has been suggested that folic acid may be concerned in the metabolism of purines and pyrimidines and in the production of nucleoprotein Its action is certainly inter-connected with that of another important anti anæmia factor vitamin B_{12} (see below p 202) but the interrelation between these two substances is not yet fully understood

STREPOGENIN

Strepogenin is the name given by Woolley (1941) to a growth factor needed by certain hæmolytic streptococci It is a peptide-like substance which occurs in association

* See Addendum, p. 5.

with various proteins but is absent from egg white. In the molecule of insulin it appears to be present at the end of the peptide chain Strepogenin is said to be a growth promoting factor for rats, mice, and guinea pigs, but has, as yet, been relatively little studied Studies with micro-organisms suggest that strepogenin may be elaborated by some enzyme system concerned with the synthesis of peptides

ANIMAL PROTEIN FACTOR(S)

Several groups of investigators, in the U.S.A. and elsewhere, have observed that their experimental animals, kept on special purified diets, thrived better when provided with small supplements of certain crude animal proteins, e.g a suitable preparation of casein Such an effect has now been reported for various species (e.g rats chicks monkeys dogs) and for various dietary supplements as well as for casein (e.g liver extracts, muscle tissue fish press juice, and even cow manure[1-16]) In poultry rearing these conclusions have been found of practical importance, under some conditions in the U.S.A

It is not yet certain how many distinct factors are involved, but vitamin B_{12} (next section p 201) is certainly to be regarded as one such animal protein factor

Another point of interest is that the animal protein factor or factors may be the same as a vitamin like substance, **physin** which was postulated by Mapson as long ago as 1932 This was shown to be present in liver it promoted lactation in the rat, and also had a transmitted growth stimulating effect on the offspring

(1) Cary *et al.* (1946) (2) Hartman (1946) (3) Jaffé (1946) (4) Bosshardt *et al.* (1946) (5) Zucker and Zucker (1946) (6) Cooperman *et al.* (1945) (7) Rubin and Bird (1946) (8) Spitzer and Phillips (1946) (9) Patton *et al* (1946) (10) Cravens *et al* (1945) (11) Jaffé and Elvehjem (1947) (12) Rueggamer Sporn *et al* (1948) (13) Zucker and Zucker (1948) (14) Bird *et al* (1948) (15) Novak and Hauge (1948) (16) Rueggamer Brickson *et al.* (1948)

VITAMIN B_{12}

This the newest addition to the family of vitamins, is in some ways perhaps also the most remarkable and the most unexpected

Mary S Shorb working at the Maryland Agricultural Experimental Station set out to search for a micro-organism which would respond to the rat growth factor or factors (animal protein factor) previously described by Cary and by Hartman (p 200) She found[1] that a particular bacterium *Lactobacillus lactis* Dorner could be used in this way It needed two such factors, one present in casein (and in tomato juice and other substances) and a second in liver extracts. The first factor she called TJ and the second LLD and later vitamin B_{12}.

A continuation of the investigations[1,2,3,4] led to the notable conclusion that vitamin B_{12} is identical with the **anti anæmia factor** i e the factor effective in treating Addisonian pernicious anæmia in man This latter factor in the form of less highly purified extracts had needless to say been familiar to physiologists and physicians ever since the pioneer work of Minot and Murphy in 1926

Vitamin B_{12} has been isolated in a crystalline state[2,5] and is remarkable for containing the element **cobalt**[6,7] to which its bright red colour can doubtless be attributed. Cobalt, it may be noted in parenthesis had already been known to be of importance in animal nutrition in another connection namely as a **trace element** its deficiency being the cause of diseases endemic in farm animal in certain areas.

(1) Shorb (1947) (2) Rickes *et al* (1948, 1); (3) Shorb (1948) (4) West (1948) (5) Smith (1948 1) (6) Rickes *et al* (1948, 2) (7) Smith (1948, 2) (8) See review by Martin (1944)

As has been explained above (p 201) vitamin B_{12} is to be regarded as one of the animal protein factors and the crystalline material has indeed already been shown to be needed for the growth of chicks.*

As to its biochemical function, observations on the nutrition of micro-organisms have indicated that vitamin B_{12} may be involved in the biosynthesis of purines and of thymidine, and also that there exists some interrelation between it and the action of folic acid (cf also p 199)

Clinical Trials of Vitamin B_{12}

In experimental clinical trials, in Addisonian pernicious

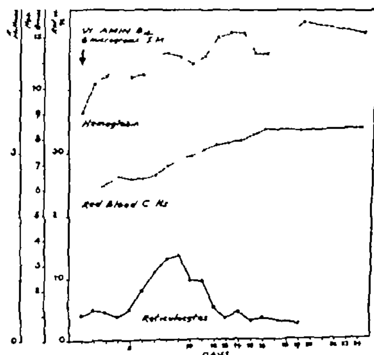


FIG 81 Hæmatopoietic response to vitamin B_{12}. Effect of injection of vitamin B_{12} in patient with nutritional macrocytic anemia.

(After Spies et al., 1948)

* Ott et al. (948)

helps to potentiate the activity of the extrinsic factor (=vitamin B_{12}) when the latter is given by mouth *

Some Properties of Vitamin B_{12}

At the time of writing the constitution of vitamin B_{12} has not yet been fully unravelled Several different forms are recognized, vitamin B_{12a}, vitamin B_{12b}, etc An interesting development is the use of a micro-organism, *Streptomyces griseus* as an alternative raw material in place of liver, for the commercial isolation of the vitamin

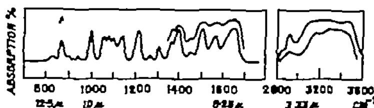


FIG 82. Infra red absorption spectrum of vitamin B_{12}. A single microscopic crystal, weighing about 10^{-6} g. was used. (After Barer 1948)

This promises to furnish increased amounts at a lower cost. In Britain, vitamin B_{12} is now sold under such trade names as anacobin, and in U.S.A. as cobione The single dose needed for full remission in pernicious anæmia has been estimated at 100 μg if injected daily a dose of 3 μg is suggested during the first six weeks of treatment, followed by a maintenance dose of 1 μg daily

Vitamin B_{12} and Folic Acid in Treatment of Macrocytic Anæmias

From what has already been said (pp 198, 203) it will be apparent that administration of folic acid is a correct treatment for some megaloblastic anæmias, notably for sprue Folic acid is however not permissible in

* See Addendum, p. 225.

the treatment of some other types of megaloblastic anæmia, particularly pernicious anæmia, because (as mentioned on p 203) it will not cure the neurological complications, and may even accelerate their appearance One recent writer has estimated that there is an 80 per cent risk of patients with pernicious anæmia developing disease of the central nervous system if kept for a long time on folic acid even when initially free from this complication

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CHAPTER XI

DIETETICS AND APPLICATIONS

Fortunately it is possible to formulate a few simple rule which should ensure the provision of an adequate supply of vitamins under most conditions—e.g with normal middle-class dietaries in Western Europe and U S.A.

Infants

For artificially fed infants the diet may be considered satisfactory—assuming the calorie requirement to be satisfied—provided (1) that it is based on whole milk (modified if necessary and with the addition of iron) and (2) that it is supplemented with orange juice or ascorbic acid and with vitamin D e.g in the form of a concentrate. Even for breast fed infants it may be a useful precaution to furnish such supplements

The daily requirements for the various vitamins have already been dealt with and generally the directions given with any reputable proprietary preparation can safely be followed. Experience in America suggests that it is possibly an advantage to provide a supplement of vitamin B_1 (or B complex) from a fairly early age this may be given as a cereal (e.g wheat germ) preparation or as a concentrate.

Diets of Adults and Children Inter war Period

The commonest faults in the diets of adults in Great Britain during the period between the two World Wars

maize is the staple diet, the public authorities have instituted measures to combat pellagra. These are but a few examples

War time and Post war Dietaries

During the Second World War years (1939–1945) measures taken by the British Government, such as the substitution of the national loaf" for the ordinary pre war white bread the vitaminization of margarine and the provision of vitamin concentrates for mothers and babies played a large part in ensuring an adequate diet. With the restrictions in the supply and in the variety of food stuffs available, analysis showed that the British population was relying to a surprising extent on a relatively small number of foods for its daily supply of each of the essential vitamins

Vitamin B_1 for example, was supplied largely by the national loaf" and to a less extent by potatoes there were no other important sources of B_1 in the diet.

Again the only important sources of vitamin C for adults were home-grown potatoes and green vegetables and to a less extent native summer fruits the importation of foreign fruits (especially the citrus varieties) had largely ceased For babies the concentrates of vitamin C (synthetic ascorbic acid preserved orange and black-currant juice) provided through Government action were the safeguard

Vitamin A, in turn was derived mostly from the vitaminized margarine, green vegetables and carrots, with smaller amounts from the milk and butter Other foods provided almost none (except for fish liver oil or concentrates for mothers and babies)

For vitamin D reliance was placed on vitaminized margarine, or for infants and mothers, on synthetic or fortified preparations.

Some of these conditions still apply (1951) although for example oranges are now again in supply

Food Consumption Levels

A report on Food Consumption Levels in the United Kingdom issued by the Ministry of Food in 1947 draws attention to the progressive increase in the intake of milk since 1939 The consumption of potatoes and of fish similarly increased while that of sugar and tea declined because, no doubt of restrictions Some of these trends in terms of foodstuffs and of nutrients are shown in Figs 83 and 84

Standards

The League of Nations standards of requirements for an adult are as follows

Vitamin A	3000 international units per day
Vitamin B_1	300
Vitamin C	30 milligrams

The prophylactic dose of vitamin D for a child is given as 500 international units per day

The foregoing figures are accepted as the normal daily requirement in a schedule to the British *Labelling of Food Order* which gives the following in addition —

Riboflavin	1.8 milligrams per day
Nicotinic acid	10

Some American standards are considerably higher than those cited above but they are defined as the recommended allowances and are to be taken as target values or desirable intakes rather than as requirements with a sufficient margin for safety which the League of Nations standard probably do very fairly represent

Civilian Consumption Per Head Per

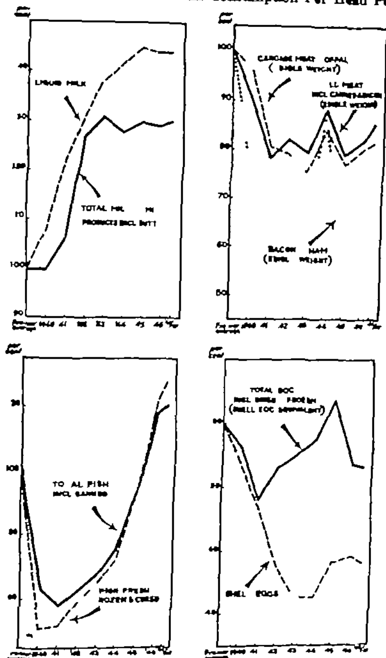


Fig 85. Food consumption levels in the United Kingdom.

The curves show that during the Second World War the consumption by civilians of butter tea, sugar, shell eggs, bacon and ham declined

Year as Per Cent of Pre-war

greatly as compared with the pr[illegible] consumption [illegible] milk [illegible] of potatoes rose [illegible]

[illegible]

Nutrient Equivalents of Civilian Consumption Per Head Per Day

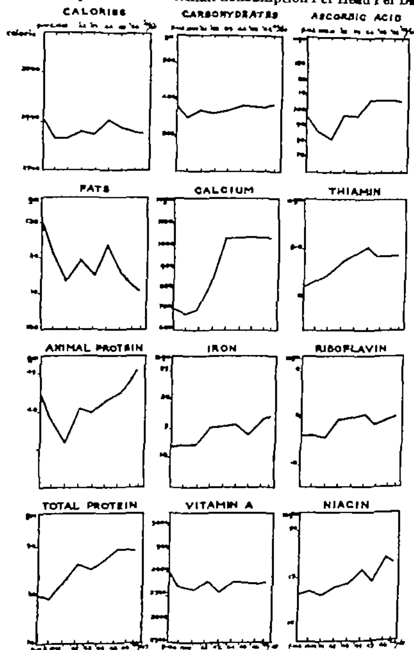


FIG 84. Food consumption levels in the U.K. in terms of nutrients. During the war years, notwithstanding the scarcity of some foodstuffs, the average consumption of the more important nutrients was still well maintained.

(From Food Consumption Levels in the United Kingdom, Ministry of Food, London, 1947 (Cmd. 7203) by courtesy of H.M. Stationery Office)

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APPEN

List of the Better

(1) Name			(2) Whether Fat-soluble or Water Soluble	(3) Corresponding Deficiency Disease in Man [or Animals]	(4) Empirical Formula	(5) Chemical Name
Vit. A			Fat-soluble	Xerosis	$C_{20}H_{30}O$ $C_{40}H_{56}$	Axerophthol β Carotene *Other A vits* Other act carotenoids
D				Rickets	$C_{28}H_{44}O$ $C_{27}H_{44}O$	Vit. D_2, calciferol Vit. D_3 Other "D" vits.
E				[Sterility]	$C_{29}H_{50}O_2$ $C_{28}H_{48}O_2$ $C_{28}H_{48}O_2$ $C_{27}H_{46}O_2$	α-Tocopherol β Tocopherol γ-Tocopherol δ-Tocopherol
K				*Hypoprothrombinaemia*	$C_{31}H_{46}O_2$ $C_{41}H_{56}O_2$ $C_{11}H_8O_2$	*Vit. K_1* Vit. K_2 Vit. K_3 (Menaphthone menadione) Other active "phylloquinones" (2 Me-1,4-naphthoquinone *derivatives*)
B	B_1		Water soluble	Beri-beri	$C_{12}H_{17}ON_4SCl.HCl$	Aneurin (*or* thiamine)
	B_2	P P		Pellagra	$C_6H_6ON_2$	Nicotinamide (*or* niacin amide)
		Riboflavin‖		(Angular stomatitis)	$C_{17}H_{20}O_6N_4$	Riboflavin
		Vit. B_6		["Rat pellagra"]	$C_8H_{11}O_3N$ $C_8H_9O_3N$ $C_8H_{12}O_2N_2$	Pyridoxine Pyridoxal Pyridoxamine
		Pantothenic acid		["Chick pellagra"]	$C_9H_{17}O_5N$	Pantothenic acid
		Other B_2 factors¶				
C				Scurvy	$C_6H_8O_6$	Ascorbic acid

Denotes that in *humans* the deficiency disease is less well recognized.

† For additional estimates of requirements, U.S.A. standards, etc. see letter press.

‡ Relatively more is needed during lactation and pregnancy.

DIX I

Known Vitamins

(6) C[illegible] [illegible] Component	(7) S[illegible] International Unit (I.U.)	(8) Approximate [illegible] Dose		(9) Approximate Daily Requirement for [illegible]	
		[illegible]	[illegible]	Adult	Infant
	0.[illegible] μg [illegible]	2.5 [illegible]		} [illegible]	
	0.[illegible] μg	1.4			400 I.U.
	1.0 mg	1 mg			
Co-carboxylase		[illegible]		[illegible]	
Pyridine dinucleotides		[illegible]	[illegible]		
Flavin dinucleotides		1 [illegible] μg			
		[illegible] μg			
Co-decarboxylase					
Acetylation co-enzyme		[illegible] [illegible] mg			
	[illegible] mg		[illegible] mg	[illegible]	

[illegible] depends on [illegible]
[illegible] [illegible] [illegible] [illegible] Vitamin [illegible]
[illegible] below [illegible] [illegible] acetate

APPENDIX II

List of Additional Vitamins Growth Factors etc.

NAME	NOTES
I *Known by letter*	
["B$_3$"]	[Rat factor] } Undefined.
["B$_4$" "B$_5$"]	[Pigeon factors] } Names now obsolete.
B_c	*See* Anti-anæmia factors, *below*
*"F" (= Nutritionally essential unsaturated fatty acids)	Linoleic acid ($C_{18}H_{32}O_2$) Linolenic acid ($C_{18}H_{30}O_2$) Arachidonic acid ($C_{20}H_{32}O_2$)
H (= 'Haut Faktor')	BIOTIN ($C_{10}H_{16}O_3N_2S$)
L (= Lactation factor)	
M (= Monkey factor)	
P (= Permeability factor)	HESPERIDIN ($C_{28}H_{34}O_{15}$) and related FLAVANONE glycosides
W (= Water-soluble rat factor)	
II *Known by name*	
BIOTIN	See above
CHOLINE	$C_5H_{15}O_2N$ (Biological transmethylating agent)
INOSITOL	$C_6H_{12}O_6$ (= Bios I)
p-AMINOBENZOIC ACID	$C_7H_7O_2N$ (Antagonized by sulphonamide drugs)
Anti-anæmic factors —	
FOLIC ACID	*Various PTERIN derivatives, e.g.*† — pteroyl-glutamic acid ($C_{19}H_{19}O_6N_7$) pteroyl-triglutamic acid ($C_{29}H_{33}O_{12}N_9$) pteroyl-heptaglutamic acid ($C_{49}H_{61}O_{24}N_{13}$)
VITAMIN B_{12}	Anti pernicious-anæmia factor of liver. Contains the element, Co
ANIMAL-PROTEIN FACTOR(S)	
STREPOGENIN	
"BIOS"	Growth-promoting factors for yeast
AUXINS	Accessory growth factors for plants
ANTI VITAMINS	Structural homologues blocking vitamin action other types of vitamin inhibitors and enhancers

*"Vitamin F" is fat-soluble; the remainder in this table are water-soluble

†For further synonyms, see letterpress

APPENDIX III

Some Important Sources of the Better Known Vitamins

Name of Vitamin	Principal Sources (with approximate activity)	Potency (I.U. or mg. per 100 grams)*
Fat-soluble group		
Vitamin A† (a)	Halibut-liver oil	3,000,000–15,000,000 I.U.
	Cod-liver oil	[illegible]0,000–200,000
	Liver (calf or ox)	5,000–15,000
(b)	Butter	2,000–5,000
	Margarine (vitaminized)	2,000
(c)	Red-palm oil	[illegible]0,000–200,000
	Carrot	10,000–20,000
	Spinach (green leaf vegs.)	5,000–20,000
D	Tunny-liver oil	[illegible],000,000–6,000,000 I.U.
	Halibut-liver oil	100,000–500,000
	Cod-liver oil	[illegible],000–30,000
	Herring-body oil	[illegible]0,000–20,000
	Cacao-shell oil	30,000
	Egg yolk	200–400
	Margarine (vitaminized)	200
	Butter	50–100
E	Wheat-germ oil	250 mg.
	Rice-germ oil	100
	Cotton-seed oil	[illegible]00
	Green leaves	5
Vitamin-B complex		
B_1	Dried brewers' yeast (edible food yeast)	1,000–2,000
	Barley germ	1,[illegible]00
	Wheat germ	500–[illegible],000
	Rice bran	500
	Oatmeal	100–200
	Wheat (whole grain)	[illegible]00–200
	Wholemeal bread	[illegible]00
	Peas	100
	Haricot beans	[illegible]00
	Egg yolk	100
	Bread, national (85 per cent extraction)	60

* Activities may vary considerably from specimen to specimen, and these figures should be taken as representative of an average range of values.

† Vitamin A. Substances in the group marked (a) contain vitamin A without carotene; those in group (b) contain vitamin A plus carotene; those in group (c) owe their vitamin A activity to carotene alone (see p. 187).

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NAME	NOTES
I *Known by letter*	
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[B_3 B_5]	[Pigeon factors] } Names now obsolete.
B_7	*See* Anti-anaemia factors, *below*
*"F" (= Nutritionally essential unsaturated fatty acids)	Linoleic acid ($C_{18}H_{32}O_2$) Linolenic acid ($C_{18}H_{30}O_2$) Arachidonic acid ($C_{20}H_{32}O_2$)
H (= "Haut" Faktor) L (= Lactation factor) M (= Monkey factor)	BIOTIN ($C_{10}H$ O_3N_2S)
P (= Permeability factor)	HESPERIDIN ($C_{28}H_{34}O_{15}$) and related FLAVANONE glycosides
W (= Water-soluble rat factor)	
II. *Known by name*	
BIOTIN	See above
CHOLINE	C_5H O_2N (Biological transmethylating agent)
INOSITOL	$C_6H_{12}O_6$ (= Bios I)
p-AMINOBENZOIC ACID	$C_7H_7O_2N$ (*Antagonized by sulphonamide drugs*)
Anti-anaemic factors—	
FOLIC ACID	Various PTERIN derivatives, e.g.† — pteroyl-glutamic acid ($C_{19}H$ O_6N) pteroyl-triglutamic acid ($C_{29}H_{33}$ O N) pteroyl-heptaglutamic acid ($C_{49}H$ $O_{24}N$)
VITAMIN B	Anti-pernicious-anaemia factor of liver Contains the element Co
ANIMAL-PROTEIN FACTOR(S)	
STREPOGENIN	
BIOS"	Growth-promoting factors for yeast
AUXINS	Accessory growth factors for plants
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Name of Vitamin		Principal Sources (with approximate Activities	in I.U. or mg. per 100 grams)*
Fat-soluble group			
Vitamin A†	(a)	Halibut-liver oil	9,000,000–15,000,000 I.U.
		Cod-liver oil	50,000–200,000
		Liver, calf or ox	5,000–15,000
	(b)	Butter	2,000–5,000
		Margarine, vitaminized	2,000
	(c)	Red-palm oil	,0,000–200,000
		Carrot	0,000–20,000
		Spinach, green leaf vegs.	5,000–20,000
D		Tuna-liver oil	000,000–6,000,000 I.U.
		Halibut-liver oil	00,000–300,000
		Cod-liver oil	10,000–30,000
		Herring-body oil	0,000–20,000
		Cacao-shell oil	30,000
		Egg-yolk	200–400
		Margarine, vitaminized	200
		Butter	30–100
E		Wheat-germ oil	250 mg.
		Rice-germ oil	00
		Cotton-seed oil	00
		Green leaves	5
Vitamin-B complex			
B		Dried brewers' yeast, edible food yeast	1,000–2,000 I.
		Barley germ	,500
		Wheat germ	500– ,000
		Rice bran	500
		Oatmeal	100–200
		Wheat, whole grain	00–200
		Wholemeal bread	100
		Peas	00
		Haricot beans	100
		Egg yolk	100
		Bread, national (85 per cent extraction)	60

* Activities may vary considerably from specimen to specimen, and these figures should be taken as representative of an average range of values.

† Vitamin A. Substances in the group marked (a) contain vitamin A without carotene; those in group (b) contain vitamin A plus carotene; those in group (c) owe their vitamin A activity to carotene alone. See p. 187.

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